PARENTAGE, MULTIPLE PATERNITY, AND REPRODUCTIVE SUCCESS: USING MICROSATELLITES TO STUDY SOCIAL INTERACTIONS IN TWO SPECIES OF PRAIRIE DOGS

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CHAPTER I

INTRODUCTION

Behavioral ecologists have documented the importance of social systems regarding interactions among individuals, parentage, inbreeding, and reproductive success (Chesser 1998; Dobson 1998; Dobson et al. 1998; Long et al. 1998; Pope 1998). Unfortunately, estimating these demographic characteristics based upon observational data is difficult and may be misleading. Therefore, there has been an increasing trend towards combining behavioral and genetic data to study social organization and reproductive success (Amos et al. 1993; Keane et al. 1997; Morin et al. 1994; Schenk and Kovacs 1995).

Prairie dogs (*Cynomys* sp.) are colonial, burrowing rodents that inhabit relatively open habitats throughout southern Canada, central United States, and northern Mexico (Hoffman et al. 1993; Hoogland 1995). Due to their colonial nature and diurnal activity patterns, prairie dogs have been the subject of numerous behavioral studies designed to better understand effects of social structure on genetic structure of populations (Dobson et al. 1998; Hoogland 1995; Sugg et al. 1996; Travis et al. 1996). Of the 5 species of prairie dogs (*C. gunnisoni, C. leucurus, C. ludovicianus, C. mexicanus,* and *C. parvidens*), black-tailed prairie dogs (*C. ludovicianus*) have been studied most intensively (Chesser 1983a, 1983b; Dobson et al. 1998; Hoogland 1995; King 1955). Recently, however, long-term behavioral studies have been initiated to better understand social structure and interactions among Gunnison's prairie dogs and the threatened Utah prairie dogs (*C. gunnisoni* and *C. parvidens*).

This thesis represents the beginning of long-term genetic studies of Gunnison's

and Utah prairie dogs. The overall objective of both studies was to use a combination of behavioral observations and highly variable genetic loci to document parentage within these study populations. A unique feature of these studies was the collection of extensive behavioral data at study colonies of both species for multiple years. Similarly, samples of blood were collected from most individuals for each year of the study, allowing essentially all individuals within each colony to be genotyped at 7 microsatellite loci. Once parentage was determined, these data were used to assess concordance between behavioral and genetic pedigrees and to examine frequency of multiple paternity and levels of male and female reproductive success. Behavioral data sets, such as the one currently being generated for Utah prairie dogs and the 1 already collected for Gunnison's prairie dogs, are valuable sources of information.

The following chapters address parentage and social interactions in 2 species of prairie dogs. Chapter 2 examines a population of Gunnison's prairie dogs collected from the Petrified Forest National Park, Apache County, Arizona during 1994 (n = 380). Chapter 3 examines a population of Utah prairie dogs from Bryce Canyon National Park, Garfield County, Utah, collected during 1996 (n = 147) and 1997 (n = 225). Both chapters are formatted for submission to the *Journal of Mammalogy*.

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CHAPTER II

MICROSATELLITE ANALYSIS OF A POPULATION OF GUNNISON'S PRAIRIE DOGS

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In recent years behavioral ecologists have combined behavioral and genetic data to study many demographic characteristics, such as reproductive success and inbreeding, in social animals. The main objective of this study was to perform a parentage analysis for each juvenile born in a colony of Gunnison's prairie dogs during 1994. Parentage was determined using 7 microsatellite loci and exclusion and likelihood methodologies. Once parentage was inferred, we combined behavioral and genetic data to examine frequency of multiple paternity and male and female reproductive success within the colony. Parentage was assigned to 31.0% of juveniles in the colony. The estimated frequency of multiple paternity was 27.1%. Estimates for litter size and number of juveniles sired per

male ranged from 1-5 and 1-14, respectively.

Key words: *Cynomys gunnisoni*, Gunnison's prairie dog, microsatellites, parentage

Behavioral ecologists have documented the importance of social systems regarding effects on social interactions, inbreeding, and reproductive success (Chesser 1998; Dobson 1998; Dobson et al. 1998; Long et al. 1998; Pope 1998; Sugg et al. 1996). Unfortunately, estimating these demographic characteristics based upon observational data is difficult. For example, paternity can be difficult to determine when organisms have large home ranges (Schenk and Kovacs 1995), underground copulation (Hoogland 1995; Taylor et al. 1997), underwater copulation (Coltman et al. 1998), or multiple mates per estrus female (e.g., Hanken and Sherman 1981; Hoogland 1995, 1998a; Robinson 1982). Moreover, because social organization may not reflect breeding structure, paternity may be underestimated if potential fathers are selected only from social groups containing offspring (Keane et al. 1997; Travis et al. 1996). Such difficulties have led to an increasing trend towards combining behavioral and genetic data to study social structure and demography (Amos et al. 1993; Keane et al. 1997; Morin et al. 1994; Schenk and Kovacs 1995).

Prairie dogs (*Cynomys* sp.) have been the subject of numerous studies designed to assess effects of social structure on genetic structure of populations (Dobson et al. 1998; Hoogland 1995; Sugg et al. 1996; Travis et al. 1996). Black-tailed prairie dogs (*C. ludovicianus*) have been the most intensively studied prairie dog (e.g., Chesser 1983a, 1983b; Dobson et al. 1998; Hoogland 1995), although several recent studies have focused on social structure in Gunnison's prairie dog (*C. gunnisoni*) colonies (Hoogland 1996, 1997, 1998a, 1998b, 1999; Travis et al. 1995, 1996, 1997). Gunnison's prairie dogs

occur in southeastern Utah, northwestern New Mexico, northeastern Arizona, and southwestern Colorado (Hoffmann et al. 1993). The organization of Gunnison's prairie dog towns is similar to that described for black-tailed prairie dogs. Specifically, a colony of Gunnison's prairie dogs is subdivided into smaller social units containing 3 or more adult females, their immediate offspring, and 1-2 breeding males (Hoogland 1999). However, social units within Gunnison's prairie dog towns, termed clans, are not as defined as those of black-tailed prairie dog towns. For example, although adult male Gunnison's prairie dogs may be associated with a group of females, these males may breed also with females of neighboring social units. Because female Gunnison's prairie dogs are philopatric, whereas males disperse from their natal clans, clans comprise closely related females and presumably breeding males that are unrelated to resident females (Fitzgerald and Lechleitner 1974; Hoogland 1999; Rayor 1988; Travis et al. 1995).

Female Gunnison's prairie dogs become sexually mature during their 1st year, whereas, males usually do not become sexually mature until their 2nd year (Hoogland 1997; Rayor 1985, 1988). Females enter a 1 day estrus once a year and may mate with 3 or more males (Hoogland 1998a, 1998b). Average litter size is about 4 (Hoogland 1998a; Longhurst 1944). Hoogland (1998a) found that litter size was dependent on a female's body size during breeding and number of mates. Offspring mortality for males and females in the 1st year is about 50%. Among individuals surviving the 1st year, females may live to greater than 6 years but most males do not survive greater than 5 years (Hoogland 1999).

In studies combining DNA fingerprinting with behavioral observations on

Gunnison's prairie dogs, Travis et al. (1995, 1996, 1997) concluded that female philopatry, male dispersal, and low levels of gene flow were important components of social structure. In 1 colony, Travis et al. (1996) found multiple paternity in 33% of litters and that 61% of all offspring were sired by males outside their natal territory. Travis et al. (1996) concluded that social groups were a poor reflection of mating groups and that knowledge of parentage, though difficult to determine, is important for assessing social interactions.

The purpose of our study was to use both behavioral and microsatellite data to assess parentage for each juvenile born in 1994 within a colony of Gunnison's prairie dogs. Once parentage was determined, these data were used to assess frequency of multiple paternity and levels of male and female reproductive success within the colony.

Microsatellites are codominant markers that have been used to determine parentage, especially paternity, in a variety of animals including barn swallows (*Hirundo rustica*---Primmer et al. 1995), chimpanzees (*Pan troglodytes*---Morin et al. 1994), horses (Marklund et al. 1994), canids (Binns et al. 1995), grizzly bears (*Ursus arctos*---Craighead et al. 1995), American bison (*Bison bison*---Mommens et al. 1998), armadillos (*Dasypus novemcinctus*---Prodohl et al. 1998), harbour seals (*Phoca vitulina*---Coltman et al. 1998), and rhesus macaques (*Macaca mulatta*---Kanthaswamy and Smith 1998). Microsatellites have been used also to determine reproductive success (Coltman et al. 1998; Craighead et al. 1995), mutation rate (Craighead et al. 1995; Keane et al. 1997), and genetic variation within and among populations (Dumas et al 1998; Paetkau and Strobeck 1994; Paetkau et al. 1995; Simonsen et al. 1998; Van Den Bussche et al. 1999).

MATERIALS AND METHODS

Behavioral data and blood were collected from essentially every individual in a Gunnison's prairie dog town (Petrified Forest National Park, Apache County, Arizona) for each year from 1989 to 1995. Methods of capture, blood sampling, and collection of behavioral data essentially follow that described by Hoogland (1995, 1997). For this study, genetic analyses were performed on all adults and juveniles collected in 1994 (n = 380). Samples from 1994 were selected because, at the time the genetic study was performed, they were the only samples available for analyses.

Genomic DNA was extracted from about 50 µl of whole blood following the methods of Longmire et al. (1997). Seven microsatellite loci were amplified via the polymerase chain reaction (PCR) with previously published primers developed by Stevens et al. (1997) from Columbian ground squirrels (*Spermophilus columbianus*). Although Stevens et al. (1997) reported 6 of the 9 primer pairs amplified a single locus in black-tailed prairie dogs, primers for all 9 loci were redesigned to allow multiplex gel loading (Table 1).

PCR amplifications were conducted in 15 µl volumes containing 50 ng of genomic DNA, 10 pmols of each primer, 9 µl True Allele Premix (Perkin-Elmer Applied Biosystems, Foster City, California), and 3.8 µl ddH_2O. The thermal profile consisted of a 12 min denaturation and enzyme activation cycle at 95°C; 10 cycles of 94°C for 15 s, 55°C for 60 s, 72°C for 30 s; followed by 25 cycles of 89°C for 15 s, 55°C for 60 s, 72°C for 30 s. A final 72°C incubation for 30 min was used to ensure that all reactions had gone to completion. For samples of DNA that did not amplify after repeated attempts using the above temperatures, we tried 1 or more of the following: 1) original sample was redialyzed in 1 X TE (Tris, EDTA) for 2-3 days to remove potential inhibitors, 2) new

sample was extracted from the same individual collected in a different year, 3) original samples reamplified at lower annealing temperatures in the 10 step cycle (52°C or 50°C). Variation at individual microsatellite loci was visualized using a Perkin-Elmer Applied Biosystems 377 Automated DNA Sequencer. Amplicons for each locus from a single individual were mixed (0.5 µl of each PCR product) and 1 µl of this mixture was combined with 3 µl of loading mix (2.5 µl of formamide, 0.5 µl of ROX size standard, 0.25 µl of loading buffer containing blue dextran). The mixed PCR---loading mix solution was denatured at 95°C for 5 min and 1.5 µl was loaded into a single lane of a 5% polyacrylamide gel. All juveniles were run on the same gel with potential mothers and fathers. For most loci, all individuals were genotyped twice to ensure accurate and repeatable genotyping. Genotypes were visualized using GENESCAN and GENOTYPER software.

Data Analysis

Observational data. -- Observational data were used to provide preliminary estimates of parentage. Maternity was assigned observationally by capturing juveniles upon first emergence from their natal burrows and assigning maternity or potential maternity to all adult females observed using the burrow. Potential fathers were assigned to each juvenile by observing which males displayed any behaviors indicative of copulation (Hoogland 1997) with the female(s) guarding a particular burrow. Observational data were used to limit the number of potential parents for microsatellite analysis.

Marker analysis. -- Unless otherwise mentioned, we used CERVUS 1.0 (Marshall

et al. 1998) for all molecular analyses including computation of allele frequencies, expected and observed heterozygosity, frequency of null alleles, polymorphic information content (PIC---index of variability for a locus), deviations from Hardy-Weinberg Equilibrium, and 2 exclusion probabilities for parentage assignment for each locus separately and all 7 loci combined. Probability of identity (PI---probability of randomly selecting 2 individuals with identical genotypes from a population) for each locus and for all variable loci were calculated as described by Paetkau and Strobeck (1994).

Maternity. -- Maternity was initially assigned based on behavioral observations. Lactating females typically guard their burrows from all other females (Hoogland 1995, 1997). Behavioral ecologists can thus assign maternity to the female guarding and using the burrow from which juveniles emerge. In 1994, burrows were limited so that some females shared burrows (see also Rayor 1988). In these cases, all females observed using a burrow were considered potential mothers of juveniles emerging from that burrow.

Maternity was also assigned using exclusion and likelihood methodologies. Each method was initially used separately by considering only those adult females determined to be potential mothers based on observational data. In exclusion approaches, adult females were excluded as potential mothers if they had any mismatches that could not be explained by null alleles. Exclusionary comparisons between candidate mothers and juveniles were performed by sight of the investigator. The female that had no mismatches with a juvenile was assigned as the mother. Maternity was also analyzed based on allele frequencies, delta criterion, and likelihood methodologies using CERVUS 1.0 (Marshall et al. 1998). Delta criterion were computed in simulation runs using data-

specific parameters. The simulation model is described in Marshall et al. (1998). Number of simulation cycles was set at 100,000 to decrease variation in delta criterion. Proportion of loci genotyped, 0.923, was determined using the marker analysis function of CERVUS. Proportion of loci mistyped was set at 0.030 to account for any errors such as mutations or null alleles. A total of 84 adult females were located in the study population, but only 74 were genotyped at 3 or more loci. Therefore, the proportion of candidates sampled was set at 88.1%. Delta criterion were calculated at 95%, 80%, 65%, and 50% confidence levels.

Each potential mother was assigned an LOD score (likelihood ratio---likelihood of the candidate mother, not a randomly selected female, being the actual mother) and the 2 most-likely mothers, those with the highest LOD scores, were used to calculate ΔLOD (ΔLOD = LOD of most-likely female minus LOD of next most-likely female). Both number of mismatches between juveniles and potential mothers and ΔLOD scores were considered when assigning maternity. The individual with the highest LOD score was assigned as the mother of the juvenile. Adult females must be genotyped at 3 or more loci to be considered valid for analysis by CERVUS. Adult females genotyped at less than 3 loci were automatically assigned an LOD of 0.

Paternity. -- Regarding behavioral assignment of paternity, Gunnison's prairie dogs possess 6 specific behaviors indicative of copulation (Hoogland 1997). Potential fathers were assigned to each juvenile by observing which males displayed these behaviors with the female(s) guarding a particular burrow.

Paternity was also assigned using exclusion and likelihood approaches. Exclusion and likelihood analyses were performed following the methods described for maternity

assignment. For likelihood methods, number of cycles, proportion of loci genotyped, and proportion of loci mistyped were identical to maternity parameters. A total of 33 adult males were found in the population. To account for the possibility that some males were not sampled, number of candidate males was entered as 43 and proportion of candidates sampled was set at 76.7%.

Parentage. -- Only those females and males not excluded as potential parents in maternity and paternity analyses were used in this analysis. For all analyses, adult females were considered the known parent and adult males were considered the candidate parent. For each juvenile, all possible combinations of candidate mothers and candidate fathers were considered.

Using exclusion methods, mother-juvenile dyads were compared to candidate fathers by sight of the investigator. Paternity was assigned only to adult males that had no mismatches with mother-juvenile dyads. Any mismatches resulted in removal of males for parentage. Parentage was assigned to the male and female that had no mismatches with each other or the juvenile.

Using likelihood methods, mother-juvenile dyads were compared to candidate fathers and parentage was assigned based on LOD scores. Simulation parameters used for parentage analyses were the same as those for paternity analyses. Each candidate father was assigned an LOD score and the 2 most-likely males, those with the highest LOD scores, were used to calculate ΔLOD. Both number of mismatches between mother-juvenile-father triads and ΔLOD scores were considered when assigning parentage. Parentage was assigned to the male-female pair with the highest LOD score.

Multiple paternity. -- Once parentage was determined, juveniles were assigned to

litters based on maternity. Those juveniles with undecided maternity were not assigned to litters and were not included in the multiple paternity analysis. Multiple paternity calculations considered only those litters that had 2 or more juveniles. A litter was considered to be multiply sired when at least 2 juveniles had different fathers or when at least 2 juveniles had different potential fathers remaining. Multiple paternity was calculated simply as the number of litters sired by 2 or more males divided by the total number of litters with 2 or more juveniles.

Reproductive success. -- Male and female reproductive success was calculated as the number of juveniles sired and number of juveniles per litter, respectively.

RESULTS

Based on observational data, the study colony consisted of 20 clans containing 84 adult females, 33 adult males, and 263 juveniles. All 380 individuals were genotyped for at least 1 locus except 2 adult females (4188 and 1993-64). Of the 378 individuals genotyped, 306 (81.0%) were scored at 7 loci, 22 (5.8%) at 6 loci, 14 (3.7%) at 5 loci, 13 (3.4%) at 4 loci, 15 (4.0%) at 3 loci, and 8 (2.1%) at 1 or 2 loci. All 8 individuals genotyped only at 1 or 2 loci were adult females as were the majority of those genotyped at only 3 or 4 loci (11 and 9 individuals, respectively). Thirty-two of 84 (38.1%) adult females were genotyped for all 7 loci. Twenty-six of 33 adult males (78.8%) were genotyped at 7 loci. One adult male was genotyped at only 4 loci (4111) and 3 were genotyped at only 3 (466, 4174, and 4182). No adult males were genotyped at fewer than 3 loci. Only 1 juvenile (4194) was genotyped at 3 loci and 3 juveniles (4186, 4219, and 4245) were genotyped at 4 loci. The remainder of the juveniles were genotyped at 5 or more loci with 248 of 263 (94.3%) being genotyped at 7 loci. No juveniles were

genotyped at less than 3 loci.

Markers. -- Numbers of alleles per locus ranged from 2-6 with a mean of 4.29. Based on PIC and PI, loci GS08, GS14, and GS22 were most informative (Table 1) and loci GS17 and GS20, both of which possessed only 2 alleles (Table 2), were least informative. For all loci, observed heterozygosity was less than expected heterozygosity (Table 1) and only locus GS26 was in agreement with Hardy-Weinberg expectations. First-parent exclusionary power was 77%. However, 2nd-parent exclusionary power, or the ability to exclude males as potential fathers when the mother was known, was 95%.

Maternity. -- Seventy-four of 84 (88.1%) potential mothers were genotyped at 3 or more loci. Maternity was initially assigned to all juveniles in the colony (excluding 2 that were removed due to lack of observational data for comparison) in the absence of data on paternity. This initial assignment was revised slightly when paternity data were added, but it was essential in assessing agreement between exclusion and likelihood methods and assigning confidence levels for maternity. It also aided in removing females not necessary for parentage analysis.

Null alleles were the apparent cause for mismatches for several mother-juvenile pairs and allowed inclusion of 105 potential mothers for 82 juveniles. Null alleles are the result of mutations that, for microsatellite loci, prevent amplification and visualization of bands on a gel. Because of difficulty in scoring locus GS22, maternity was reassessed after removing this locus and any females that were previously removed due to a mismatch at this locus were included as a potential mother. The removal of GS22 allowed the inclusion of 34 females as potential mothers for 33 juveniles. Eleven additional females, for 11 different juveniles, were included as potential mothers by a

combination of the removal of locus GS22 and consideration of null alleles.

Based on exclusion methods, maternity was assigned to a single most-likely female for 168 of 261 (64.4%) juveniles. Maternity was ambiguous (more than 1 possible female) for 64 of 261 (24.5%) juveniles and it was not assigned to 29 (11.1%) juveniles because all females were excluded as potential mothers. Using CERVUS, maternity was assigned at 95%, 80%, 65%, 50%, and "most-likely" confidence levels for 6, 17, 33, 32, and 99 juveniles, respectively. Delta LOD scores for the most-likely female ranged from less than 0.1 to 3.6 (Fig. 1). For the remaining 74 juveniles, maternity could not be assigned at any of these confidence levels for 30 juveniles (29 for which potential mothers were genotyped at fewer than 3 loci and 1 for which the 2 most-likely females had the same LOD scores) and all potential mothers were excluded for 44 juveniles.

Exclusion and likelihood methodologies were concordant in maternity assessment for 213 of 261 juveniles (81.6%). Both methods chose the same female as most-likely mother for 132 juveniles. Both methods failed to exclude the same suite of females but either chose different females as the most-likely mother (n = 18) or no comparisons could be made because determining most-likely female was not possible using exclusion methods (n = 50). For 13 juveniles, both methods excluded all possible females as potential mothers.

Paternity. -- As in maternity analyses, 2 of 263 juveniles were removed from paternity analyses due to lack of behavioral data. Concordance between exclusion and likelihood approaches occurred in paternity assessment for 192 of 261 (73.6%) juveniles. Disagreements occurred when 1 method eliminated all potential males as fathers and the

other method failed to do so, or when the 2 methods excluded different sets of males. Most comparisons between the 2 approaches were difficult to evaluate because, for 122 of 192 juveniles, it was not possible to select a most-likely father using exclusion methods. However, in all 122 instances, both methods failed to exclude the same group of perspective males. For 25 of 192 juveniles, both methods failed to exclude the same group of potential fathers but chose different males as most-likely father. For the remaining 45 juveniles, both methods chose the same male as most-likely 40 times and excluded all potential fathers 5 times.

Parentage. -- Final maternity and paternity assignments were made during parentage analyses using a combination of behavioral data and exclusion and likelihood methodologies. Parentage was assigned to 81 of 261 (31.0%) juveniles involved in the analyses (Appendix I). Parentage was assigned at 95%, 80%, 65%, and 50% confidence levels to 15, 13, 12, and 16 juveniles, respectively. Two additional juveniles were assigned parentage at the "most-likely" confidence level. Delta LOD scores for the most-likely male ranged from less than 0.1 to 4.0 (Fig. 2). For 7 juveniles, parents assigned with positive LOD scores were the most-likely combination of parents based on exclusion methods. In the remaining 16 instances, parentage was assigned as most-likely by exclusion analyses, but likelihood methods resulted in negative LOD scores. Of the 81 instances in which parentage was assigned, only 13 involved parents with no mismatches with the juvenile. This number increased to 33 when locus GS22 was removed. For the 180 juveniles for which parentage was not assigned, some individuals were removed as potential parents but exact parentage could not be assigned. For 49 of these juveniles, only maternity could be assigned and for the remainder, more than 1

mother and/or more than 1 father could have been assigned to them.

Multiple paternity. -- A total of 65 litters encompassing 193 juveniles were identified from parentage analyses. All but 6 had 2 or more juveniles allowing tests for multiple paternity in 59 litters. Sixteen of these 59 litters (27.1%) showed unequivocal multiple paternity. However, of the 59 litters in which we were able to test for multiple paternity, only 4 had all juveniles sired by the same male. In the remaining litters, we were unable to assign paternity for every juvenile because of either inability to exclude males as potential fathers or exclusion of all males as the possible father. Because of these problems, 27% is a minimal estimate of multiple paternity in this colony. The maximal estimate, which is calculated as the total number of litters that are or may be multiply sired (55) divided by the total number of litters with 2 or more juveniles (59), is 93.2%.

Reproductive success. -- Based on parentage analyses, 65 of 84 (77.4%) adult females in the colony successfully produced litters. Number of juveniles per litter for the 65 litters ranged from 1-5 with a mean of 2.97. These are probably underestimates of reproductive success and litter size in that we were only able to determine maternity for 130 of 261 (49.8%) juveniles.

Reproductive success for males was more difficult to determine. Paternity was resolved for 90 of 261 juveniles (34.5%); these 90 juveniles were sired by 18 of 33 (54.5%) adult males found in the colony. Based on paternity for these 90 juveniles, number of juveniles sired per male ranged from 1-14 with a mean of 5. For the remaining 171 juveniles, paternity was undecided.

DISCUSSION

Exclusion and likelihood methods. -- Parentage has traditionally been determined using exclusion probabilities (Chakraborty et al. 1988; Morin et al. 1994). However, several problems arise when parentage is determined with exclusion probabilities, especially when hypervariable microsatellite loci are used. To alleviate some of these problems, several authors advocate the use of likelihood algorithms (Meagher 1986; Thompson 1975, 1976). However, both exclusion and likelihood methods have advantages and disadvantages. Exclusion methods provide an estimate of exclusionary capabilities of each locus used in a study. In other words, the overall variability of a group of loci are reflected in exclusion probabilities. The exclusion probabilities computed for this study were similar to those obtained in other studies (Coltman et al. 1998; Kanthaswamy and Smith 1998; Mommens et al. 1998; Primmer et al. 1995). Exclusion methods also allow removal of individuals that are not the actual parents of a particular juvenile. However, exclusion methods require perfect matches between offspring and parents at all loci. There are several ways that mismatches are generated between offspring and true parents including null alleles, mutations, accuracy of estimating allele frequencies in the population relative to the portion of the population sampled, and errors in assigning genotypes. Exclusion methods are also unable to provide means of unambiguous assignment in many cases. In this study, where upwards of 1,300-1,400 parent-juvenile comparisons had to be made for each locus, exclusion methods also proved to be time consuming.

In contrast to exclusion methods, likelihood methods are able to account for mutations, null alleles, typing error, and missing data. These methods are also more discriminatory and are able to make assignments in cases where exclusionary methods

are not. Perhaps the biggest advantage in a large study such as this is the advent of computer programs, such as CERVUS (Marshall et al. 1998), that are able to perform parent-juvenile comparisons. There are some disadvantages to using likelihood methods. For example, likelihood approaches assume that each locus conforms to Hardy-Weinberg equilibrium and it is not clear what effect violating this assumption for different numbers of loci has on resultant parentage assignments. In this study, only 1 locus (GS26) was in Hardy-Weinberg equilibrium. Two difficulties we experienced with CERVUS were the occurrence of negative LOD scores and assignment of parentage to individuals having large numbers of mismatches with the juvenile in question. Negative LOD scores were common and were most likely due to mismatches resulting from null alleles, commonness of genotypes, and/or missing data.

In this study, assignment of parentage based on exclusion methods was weighed more heavily than that based on likelihood methods because exclusion methods tended to be more robust and conservative (prevented removal of individuals as parents). Because we had several candidate parents, especially females, that were not genotyped at all 7 loci we wanted to prevent exclusion of individuals as parents based on lack of genotypic data. Once genotypes of these individuals are completed we will use stricter methods of assigning parentage. In addition CERVUS attempted to assign parentage even when it appeared that all males and females should be excluded due to a high number of mismatches. CERVUS actually assigned parentage to a higher percentage of individuals than was predicted based on values calculated during simulation runs (Table 3). CERVUS was utilized mainly for calculating confidence values and for discerning possible mismatches due to mutations.

As with many parentage studies, parentage could not be assigned to all juveniles in this study population. Most parentage studies result in only a portion of juveniles with parentage assigned (e.g., Coltman et al. 1998; Kanthaswamy and Smith 1998; Keane et al. 1997; Petri et al. 1997; Prodohl et al. 1998). Typically, maternity is determined based on observational data and it is paternity that researchers are trying to determine. Success rates can be relatively high, for example, Kanthaswamy and Smith (1998) were able to assign paternity to 127 of 129 (98.4%) rhesus macaques. However, most studies have relatively lower success rates. Coltman et al. (1998) were only able to assign paternity to 85 of 275 (30.9%) harbour seal juveniles over a 2 year period. Similarly, Petri et al. (1997) and Keane et al. (1997) had success rates of 37% and 55%, respectively. Even when parentage could not be assigned, some potential parents could be removed. Enough parentage assignments were made in our study that certain demographic characteristics, such as multiple paternity and reproductive success, could be addressed.

Multiple paternity. -- Multiple mating by females appears to be common in several sciurids (Boellstorff 1994; Hanken and Sherman 1981; Hoogland 1995; Murie 1995), however the adaptive significance is not well understood. One possibility is that it ensures insemination of the female. Hoogland (1998a) found the probability of conception and parturition in Gunnison's prairie dogs was 100% if females mated with 3 or more males and only 92% if only 1 or 2 males were involved, thereby supporting the hypothesis that multiple mating may ensure female insemination. Females that mate with a high number of males also have larger litters than those females that only mate with 1 or 2 males (Hoogland 1998a). Another potential result of multiple mating by females is a litter that is sired by more than one male. Multiple paternity has been shown in several

sciurids including California ground squirrels (*Spermophilus beecheyi*---Boellstorff et al. 1994), Belding's ground squirrels (*S. beldingi*---Hanken and Sherman 1981), Columbian ground squirrels (Murie 1995), and black-tailed prairie dogs (Hoogland 1995). The frequency of multiple paternity reported for this colony of Gunnison's prairie dogs (27.1%) is within the range reported for these other sciurids (5-89%).

Reproductive success. -- Estimates of reproductive success, litter size, and number of juveniles sired per male for this population are most likely underestimates because parentage could not be assigned to all juveniles. Estimates of litter size for this colony, however, do fall within the range of those expected for Gunnison's prairie dogs (Hoogland 1997).

Parentage was often difficult to assign because potential mothers were genotyped at relatively few loci and some of these loci (GS17, GS22, or GS26) were not highly variable. A total of 134 juveniles were affected by lack of data (e.g., a female genotyped at 4 or fewer loci) for 1 or more potential mothers. As an extreme example, 7 juveniles (487, 489-491, and 498-4100) shared the same 3 potential mothers, all of which were genotyped at 3 or fewer loci. A second problem was that adult females were, for unknown reasons, relatively difficult to genotype, although DNA samples from adult females were extracted and assayed under the same conditions as those of juveniles and adult males. Possible explanations for the inability to genotype several females including mutations at primer annealing sites and presence of proteins or other inhibitors preventing amplification have been considered although the actual reason is not known. The lack of genetic data was not as problematic for paternity assignment. However, 2 adult males (466 and 4182) were genotyped at only 3 loci and could not be excluded in many

instances when they were potential fathers.

Another problem faced when attempting to exclude certain candidate parents was the degree of relatedness among candidates themselves. Most females in a clan are related due to their philopatric nature. This leads to difficulties if, for example, a mother and daughter are both potential mothers of the same juvenile. Similar problems arise if 2 related males are potential fathers for the same juvenile. These problems cannot be addressed in the absence of a pedigree. A pedigree for these individuals is being developed and should aid in resolving kinship.

For several juveniles, all potential parents were removed during exclusion analyses. This occurred for 29 of 261 (11.1%) juveniles during maternity assignments. In 23 of these instances (79.3%), the female removed was the only possible mother based on behavioral observations. The genetic mismatches between these juveniles and their potential mother were most likely due to mutations. For the 23 instances where there was only a single potential mother, a mutation rate could be calculated. Mutation rate can be calculated by dividing total number of mismatches between suspected mother-juvenile dyads at a particular locus by total number of alleles in the population at that locus. Suspected mutations were found at loci GS08, GS12, GS14, and GS22 and mutation rates were calculated to be 1.6×10^{-2}, 1.5×10^{-3}, 2.3×10^{-3}, and 1.7×10^{-2}, respectively, which are within the range estimated for microsatellites (10^{-2}-10^{-5}---Weber and Wong 1993).

All possible fathers were excluded for 43 of 261 (16.5%) juveniles. It is more difficult to assign paternity behaviorally because copulations may occur out of sight of the observer. Because all males were removed for these juveniles, it is possible that the

actual father either was not captured or was not listed as a potential father for these juveniles. Eighteen juveniles did have 1 potential father for which their was no blood sample taken (Appendix I).

A final problem faced in this study was the scoring of locus GS22, which when ran at lower temperatures during amplification, tended to produce split peaks known as stutter. Stutter produced peaks that were 2 base pairs apart. The stutter effect precluded accurate scoring, especially because most alleles for this locus were only 2 base pairs apart. For all analyses, locus GS22 was removed and paternity and maternity were reassessed with those males and females being excluded solely by GS22 being included as a potential father or mother.

Parentage assignment of Gunnison's prairie dogs was hampered by relatedness of candidate parents, missing genetic data, and possible mutations. An extended pedigree should aid in reducing some difficulties. Addition of new markers may also clarify certain parent-juvenile relationships. In those cases in which maternity was disputed, addition of mitochondrial markers might provide some resolution.

To address questions and problems raised by this study, a pedigree is being constructed from samples collected at the study colony (1991-1994). The development of an extensive pedigree should contribute to addressing questions pertaining to multiple paternity, reproductive success, and relatedness of interacting individuals, among others.

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Table 1. -- Locus name, PCR primers, and descriptive statistics for genetic variation at each locus for a population of Gunnison's prairie dogs (*Cynomys gunnisoni*) collected from the Petrified Forest National Park, Apache County, Arizona, 1994. A = number of alleles, n = sample size, H_O = observed heterozygosity, H_E = expected heterozygosity, PIC = polymorphism information content, PE1 and PE2 are 1st- and 2nd-parent exclusionary probabilities, respectively, and PI = probability of identity.

Locus[a]	Forward Primer[b]	Reverse Primer[b]	A	n	H_O	H_E	PIC	PE1	PE2	PI[c]
GS08	HEX-ACCAATGGGAGACACATCCAA	GTGTCTTAAACTCCTTGTAATAGCCCCCTG	5	321	0.636	0.696	0.647	0.276	0.450	0.140
GS12	NED-CCAAGAGAGGCAGTCGTCCAG	GTGTCTTTCGAGCAGAGCACTTACAGA	6	331	0.363	0.492	0.473	0.139	0.310	0.273
GS14	6FAM-CAGAATCAGGTGGGTCCATAGTG	GTGTCTTGATGAAACCTATTTGCCTTCCTTC	6	352	0.642	0.804	0.773	0.430	0.608	0.072
GS17	6FAM-CAATTCGTGGTGGTTATATC	GTGTCTTCTGTCACCTATATGAACACA	2	370	0.165	0.169	0.155	0.014	0.077	0.705
GS20	6FAM-GCCCAGCCATCACCCTCACC	GTGTCTTTCCAGAGTTTTTCAGACACA	2	327	0.076	0.134	0.125	0.009	0.062	0.759
GS22	6FAM-AGAGAACAACATCATCAACAGGGTGTG	GTGTCTTGGTCCTCATCCTGCCAATTTC	5	377	0.305	0.686	0.631	0.267	0.433	0.154
GS26	NED-GGCTCCAAGTCCCAGGGAC	GTGTCTTGGTCCTCATCCTGCCAATTTC	4	378	0.437	0.453	0.411	0.106	0.245	0.341
Mean			4.29		0.375	0.490	0.459	0.772	0.945	$7.7X10^{-5}$

[a]Locus names as those originally described by Stevens et al. (1997).

[b]Primers for these loci were redesigned based on DNA sequence data of Stevens et al. (1997).

[c]Probability of Identity was calculated following the method of Paetkau and Strobeck (1994).

Table 2. -- Allele frequencies for 378 *Cynomys gunnisoni* from the Petrified Forest National Park, Apache County, Arizona, 1994. For each locus, the allele, how many times that allele was found in the population, number of heterozygous individuals with that allele, number of homozygous individuals with that allele, and allele frequency are reported.

Locus	Allele	Count	Heterozygotes	Homozygotes	Frequency
GS08	183	4	4	0	0.0062
	187	95	79	8	0.1480
	189	133	121	6	0.2072
	193	118	90	14	0.1838
	195	292	114	89	0.4548
GS12	164	56	44	6	0.0846
	166	32	20	6	0.0483
	180	35	29	3	0.0529
	182	30	18	6	0.0453
	184	46	24	11	0.0695
	186	463	105	179	0.6994
GS14	181	139	59	40	0.1974
	183	152	94	29	0.2159
	185	74	44	15	0.1051
	187	112	94	9	0.1591
	191	37	25	6	0.0526
	193	190	136	27	0.2699

Table 2. Continued.

Locus	Allele	Count	Heterozygotes	Homozygotes	Frequency
GS17	151	671	61	305	0.9068
	170	69	61	4	0.0932
GS20	237	607	25	291	0.9281
	240	47	25	11	0.0719
GS22	130	320	70	125	0.4244
	132	250	28	111	0.3316
	134	97	51	23	0.1286
	136	22	22	0	0.0292
	142	65	59	3	0.0862
GS26	101	30	28	1	0.0397
	105	539	145	197	0.7130
	107	140	110	15	0.1852
	109	47	47	0	0.0622

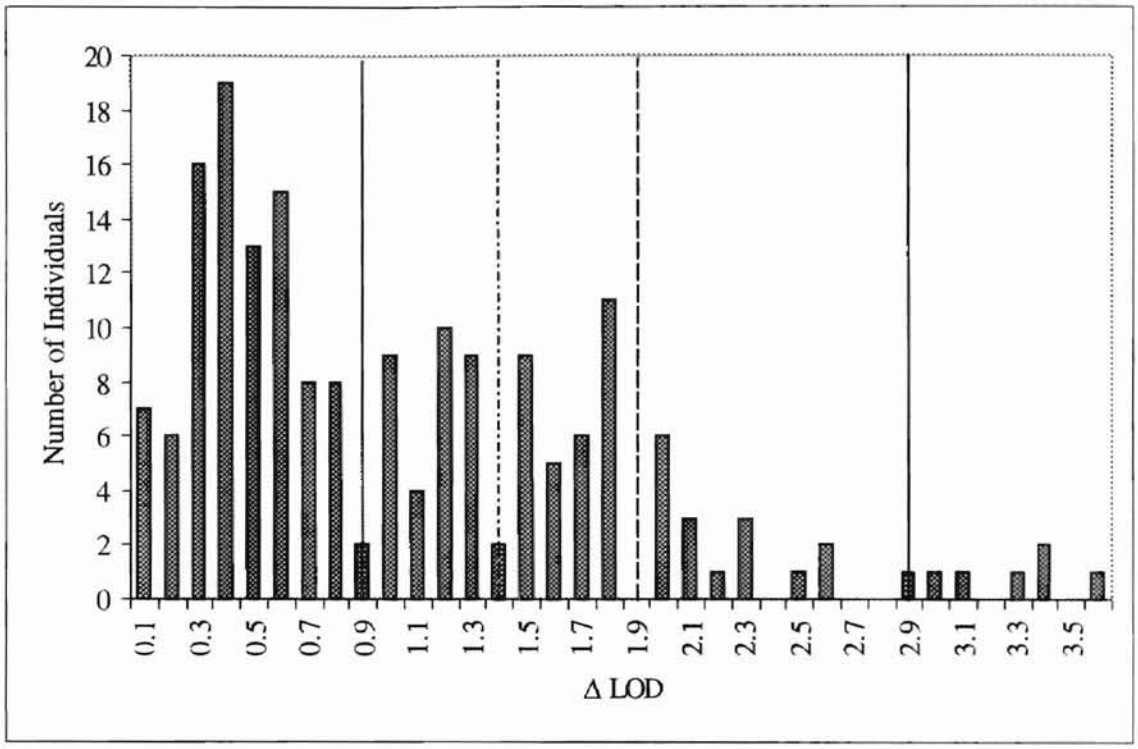
Table 3. -- Confidence levels, critical ΔLOD scores (Δ LOD = LOD of most-likely male minus LOD of next most-likely male), and percentage of predicted and assigned parentage for 261 juvenile *Cynomys gunnisoni* from the Petrified Forest National Park, Apache County, Arizona, 1994. Values for "assignments made" were averaged over 10 parentage trials (trial 1 = 1 candidate mother, trials 2-3 = 2 candidate mothers, trials 4-6 = 3 candidate mothers, trials 7-10 = 4 candidate mothers).

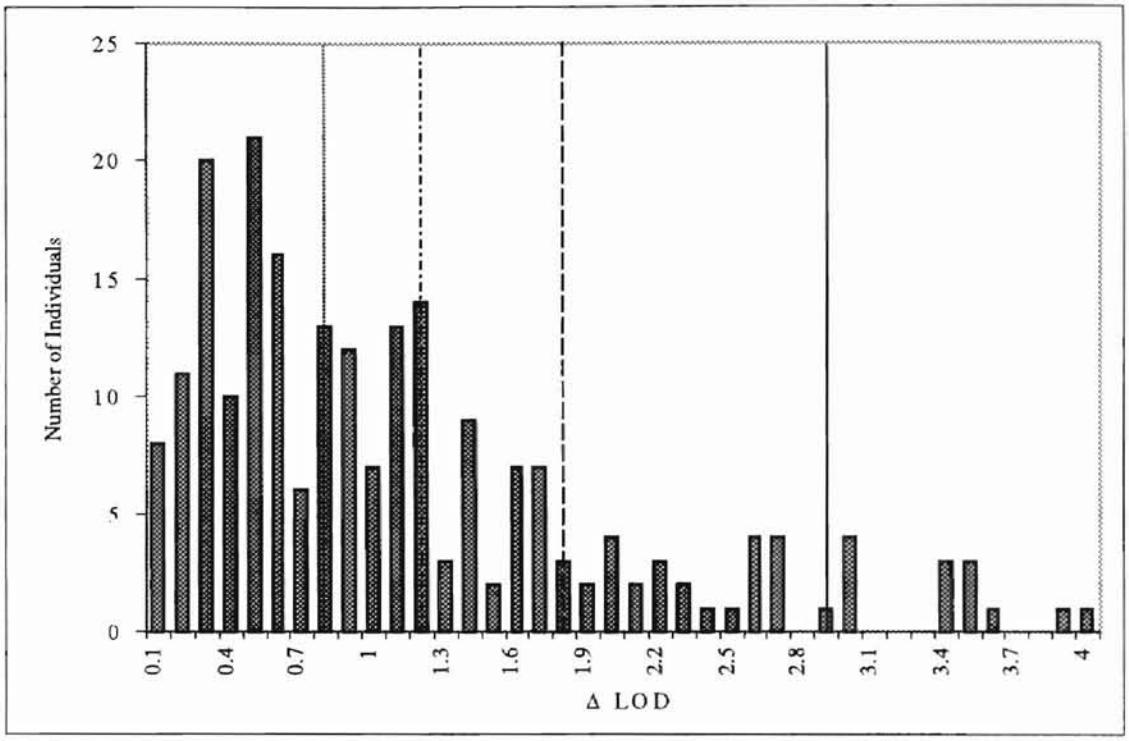
Maternity known			
Confidence level	Critical ΔLOD	Predicted assignments	Assignments made
95%	2.39	3%	3.8%
80%	1.19	15%	29.0%
65%	0.67	33%	60.9%
50%	0.28	62%	81.0%
Maternity Unknown			
95%	2.85	0%	1.6%
80%	1.80	1%	8.2%
65%	1.24	6%	25.1%
50%	0.76	18%	45.4%

FIGURE LEGENDS

Fig. 1. -- Distribution of ΔLOD scores for most-likely candidate females calculated during maternity assignments for 261 *Cynomys gunnisoni* juveniles from the Petrified Forest National Park, Apache County, Arizona, 1994. Five individuals with ΔLOD scores < 0.1 are not included. Critical ΔLOD for 95, 80, 65, and 50% confidence levels are shown with solid, dashed, dashed-dotted, and dotted lines, respectively. Calculations were made using CERVUS 1.0 (Marshall et al. 1998).

Fig. 2. -- Distribution of ΔLOD scores for most-likely candidate males calculated during parentage assignments for 261 *Cynomys gunnisoni* juveniles from the Petrified Forest National Park, Apache County, Arizona, 1994. Nine males with ΔLOD scores < 0.1 are not included. Critical ΔLOD for 95, 80, 65, and 50% confidence levels are shown with solid, dashed, dashed-dotted, and dotted lines, respectively. Calculations were made using CERVUS 1.0 (Marshall et al. 1998).


Number of Individuals
20
18
16
14
12
10
8
6
4
2
0
0.1
0.3
0.5
0.7
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
Δ LOD


Number of Individuals
25
20
15
10
5
0
0.1
0.4
0.7
1
1.3
1.6
1.9
2.2
2.5
2.8
3.1
3.4
3.7
4
Δ LOD

APPENDIX I

Parentage. -- Parentage was determined using observational, exclusion, and likelihood methods for 261 *Cynomys gunnisoni* juveniles from the Petrified Forest National Park, Apache County, Arizona, 1994. Maternity and paternity assignments listed in the table are final parentage assignments for this population. All confidence levels given were calculated by CERVUS (Marshall et al. 1998) and represent the confidence of the mother-juvenile-father triad. Juveniles marked with an asterisk (*) had 1 potential father with no blood sample. A dash (--) indicates that no mother, father, or confidence level could be assigned. An asterisk (*) beside an individual in the maternity or paternity column indicates that assignment of that parent was based on observational data only. A double asterisk (**) indicates that assignment of that parent was based on observational data supported by likelihood methods. An individual with an (n) had a mismatch with the juvenile that was due to a null allele (a mutation that prevents amplification and visualization of an allele). Individuals in [] were included as parents only after locus GS22 was removed. A (-LOD) under the confidence column indicates that the individuals selected as the parents had a "most-likely" confidence level based on exclusion methods but could not be assigned a confidence level using CERVUS. An ml indicates a "most-likely" confidence level.

Juveniles	Maternity	Paternity	Confidence
406	405**	404(n)	-- (-LOD)
407	405(n)	407	80%
408	405	--	--
410	[413]	[401]	80%

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
411	409(n)	401(n)	50%
412	409	--	--
414	409	--	--
415	409,413(n)	--	--
418	417	[404(n)]	80%
419	417	403(n)	50%
426	425(n)	--	--
427	425	424**	50%
428	425	420**	80%
430	429(n)	[423]	95%
431	429**	[423]	50%
432	429**	--	--
440	443,[448]	449(n)	--
441	439,448	[423]	--
442	448**,439**	420,[421]	--
444	439**,443**	--	--
445	443	--	--
446	443	--	--
447	443	--	--
450	439,[448]	421,[420]	--
451	443,[448]	449(n),422	--

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
452	443	--	--
454	453	422(n)	65%
455	458	422(n)	95%
456	458	422	95%
457	453	422,[420]	--
459	458	422(n),[423(n)]	--
460	458	422(n)	50%
434	1993-64(n),[433]	--	--
435	1993-64	--	--
436	433	423	65%
437	[433]	[423]	+LOD
438	433	420(n)	65%
462	461	423**	50%
463	461	423(n),[4111(n)]	--
464	461	[420(n)]	-- (-LOD)
4109	4378(n),4122	[449(n),4111(n)]	--
4110	4108,[4122,4378,4135]	420	--
4113	[4112]	[4111]	+LOD
4114	[4112]	423**	50%
4115	4112**	[4111]	+LOD
4116	4112	[4111]	--(-LOD)

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
4118	4117	420,[421]	--
4119	4117(n)	[421,420]	--
4120	4117	420,[421]	--
4121	4117(n)	[421,420]	--
4123	4135,4122	420(n)	--
4124	4129(n),4378(n), 4108(n),4122	423(n)	--
4125	4129,4135,4378, 4122	423	--
4126	4378,4122	[420]	--
4127	4378(n),4122(n)	[421,420]	--
4128	4108(n),4122(n)	[420]	--
4130	4378(n),4122(n)	420,[421]	--
4131	4108,[4122,4378]	420,[421]	--
4132	4108,[4122]	420	--
4133	4378(n),4122(n)	420,[421]	--
4134	4378(n),4122(n)	420,[421]	--
4136	4122,[4108]	[420]	--
4137	4135(n),4378(n), 4122(n)	4111(n),[423]	--
467	465	4226(n),466(n),[4182]	--
468	[465]	421,4226,466,4182, [4183]	--

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
469	465	4226(n),466,4182	--
470	465	466,4182	--
472	[471]	466,4182	--
473	471**	4182(n)	--(-LOD)
474	471**	[4182,466]	--
475	471(n)	[4182,466]	--
477	476	466(n),[4182]	--
478	476(n)	466,4182	--
479	476	466(n),[4182]	--
480	476(n)	4226(n),466,4182	--
482	481	[4226(n),4182,466]	--
483	481	466(n),[4182]	--
484	[481]	4226(n),466,4182	--
485	481	4226(n),466(n),[4182]	--
487	488(n),497(n),[486]	[4182,466]	--
489	488,497,[486]	466,4182	--
490	488,497,[486]	466,4182	--
491	488,497,[486]	[4182,466]	--
498	488,497,[486]	466,4182	--
499	488,497,[486]	466,4182	--
4100	488(n),497(n),[486]	[4226(n),466(n),4182]	--

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
493	492(n)	466,4182	--
494	492**	466(n),[4182]	--
495	492*	466,4182,[4226]	--
496	492	466,4182	--
4102	4101	466,4182	--
4103	492**	4183,[4182,466]	--
4104	4101	466,4182	--
4106	4105(n)	466,4182,[4183]	--
4139*	4138*	[402]	50%
4140*	4138*	402	95%
4141*	4138*	[402]	95%
4143*	4142	402**	65%
4144*	4142	402**	65%
4146*	4145	401(n)	50%
4147*	4145	402	80%
4148*	4145	402**	80%
4150*	4149	402**	95%
4151*	4149	402**	95%
4152*	4145,[4142]	403(n)	--
4154	4153	--	--
4155	4153	[4164]	--(-LOD)

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
4157	4153(n)	--	--
4158	4161,4156,4153	--	--
4159	4156(n),4153(n)	--	--
4160	4153	416	95%
4166	4161*	4164,[4165]	--
4167	4161*	[403(n)]	+LOD
4168	4161	[4164]	+LOD
4170*	4169**	4176,1993-63,4174	--
4171*	4169**	4174	50%
4172*	4169	1993-63,4174	--
4173*	4169**	4174	ml
4178*	4177(n)	4174,[402]	--
4179*	4169	4174	50%
4180*	4177**	4174	--(-LOD)
4184	1993-50,4188	421,466,4182,[4183]	--
4185	1993-50,4188	424(n),466,4182	--
4186	1993-50,4188,[4349(n)]	466,4182,424	--
4187	1993-50,4188	4183,[421,4182,466]	--
4189	4188*	424(n),466,4182	--
4190	4188*	424(n),466(n),[4182]	--
4191	4188*	421,466,4182,[424, 4183]	--

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
4192	4188*	424(n),466(n),[4182]	--
4194	4193	4183,4175,[424,4176, 4182]	--
4195	4193	4183	80%
4196	4193	4183,[4182]	--
4198	4197(n)	4183(n),4176(n),4182	--
4199	4197	4183(n),4182	--
4200	4197*	--	--
4201	4193,4202	--	--
4203	4202	4176,4182,[424]	--
4204	4202	4182	65%
4205	4193(n),[4202]	4182,[4183]	--
4207	4206(n)	--	--
4208	4214(n),[4210(n)]	--	--
4209	4214	[4183]	+LOD
4211	4373,4206	--	--
4212	4206(n)	--	--
4213	4214,[4210]	4182	--
4215	4214(n),[4206(n)]	--	--
4216	4214	4176,4182,[4175,424]	--
4217	4214(n),[4373(n)]	4176,4182	--
4218	4214	--	--

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
4219	4214,[4210,4373]	4176,4182,[424,4175]	--
4220	4373,4214	--	--
4222	[4221]	4227,4226,466	--
4223	4221	4226(n),466(n)	--
4224	[4221]	4226,466	--
4229	4225(n)	4227(n),466,[449,4228]	--
4230	4225*	4226,466	--
4231	4225*	4226,466	--
4232	4225*	4226,466	--
4233	4225*	4226,466	--
4235	4234(n)	4227,4226,466	--
4236	4234	4226(n),4227(n),466(n)	--
4237	4234	466(n)	--(-LOD)
4239	4246,4238,4254	4227,466	--
4240	4238(n),4254(n)	[4227,466]	--
4241	4238(n)	4227,466,[449,4228]	--
4242	4238(n)	4227,466,[449,4228]	--
4244	4243*	4226,466	--
4245	4243*	4226,466	--
4247	4254(n),[4246(n)]	449,4228,[466]	--
4248	4254	4227(n),466	--

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
4249	4238(n)	4227,466,[449,4228]	--
4251	[4250]	[466]	--(-LOD)
4252	4250	4227,466	--
4253	4250	4227(n),466	--
4255	4238,4254	4227,466	--
4256	4238(n)	449(n),4228(n), 4227(n),466	--
4258	4262,4243(n),4267(n)	[4226,466]	--
4259	4225,4262,[4257,4243]	[4226,466]	--
4260	4225,4262,[4257,4243]	[4226,466]	--
4263	4262(n)	4226,466	--
4264	4262(n)	4226,466	--
4265	4262	4226,466	--
4267	4266	4228**	65%
4268	4266	4228**	65%
4269	4266	--	--
4271	4270	401	95%
4272	4270(n)	--	--
4273	4270	401(n)	80%
4275	4274	[401(n)]	--(-LOD)
4276	4274**	[401]	50%
4277	4274	[404(n)]	50%

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
4278	4274	404(n)	50%
4280	4279	--	--
4281	4284**	--	--
4282	[4279]	[403]	65%
4283	4284	--	--
4285	4284	404(n)	95%
4286	4284(n)	--	--
4288	4287	4228(n),416(n),401(n)	--
4290	4289(n)	--	--
4291	4289(n)	404(n)	95%
4292	4289(n)	404(n)	80%
4293	4289	404(n)	--
4295	4294	403(n),416(n),4164(n)	--
4296	4294	416(n)	--(-LOD)
4297	4294	416(n),4299(n)	--
4298	4294	[403]	65%
4301	4300*	416	95%
4302	4300*	416	95%
4303	4300*	416	50%
4304	4300*	416(n),[4164(n)]	--
4305	4300*	416	95%

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
4310	4306	423(n)	ml
4311	4306	449	80%
4312	4306(n)	--	--
4313	4306(n)	--	--
4315	4314	449(n)[4309(n), 4307(n)]	--
4316	4314	--	--
4318	4317	[449]	65%
4319	4317	4307(n)	80%
4320	4317	--	--
4322	4321	--	--
4324	4323(n)	[423(n)]	+LOD
4325	4323	423**	65%
4326	4323	--	--
4328	4327*	[423(n)]	--(-LOD)
4329	4327(n)	--	--
4330	4327	--	--
4332	4331*	--	--
4333	4331(n)	--	--
4335	4334(n)	449(n),[423(n)]	--
4336	4334(n)	[423(n)]	--(-LOD)
4337	4334(n)	[423(n)]	80%

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
4339	4338(n)	--	--
4341	4345,[4340]	4226**	--
4342	4345,4340	4226**	--
4343	4340,[4345]	--	--
4344	4340,4345	--	--
4346	4345,[4340]	4226**	--
4347	4340,[4345]	--	--
4348	4340,[4345]	--	--
4350	4359,4353,4349	[4182]	--
4351	4359*	424,420,[4182]	--
4352	4353,4359	424,[4182]	--
4354	4359,[4353]	424,4182,[421,420]	--
4355	4359,[4353,4349]	4182	--
4356	4353	[4182]	--(-LOD)
4357	4353	[4182]	--(-LOD)
4358	4353	424,[4182]	--
4360	4359*	424.[420,421,4182]	--
4361	4359,4349(n)	[4182]	--
4362	4359*	[4182]	--(-LOD)
4364	4363	4183,[466,4182]	--
4365	4363	4183(n)	95%

APPENDIX I. Continued.

Juveniles	Maternity	Paternity	Confidence
4366	4363	4183,[4182,466]	--
4368	4367	449(n),4111,[423(n)]	--
4369	4367(n)	4111	80%
4370	4367(n)	[423(n)]	--(-LOD)
4371	4367	--	--
4372	4367	423(n)	50%

CHAPTER III

MICROSATELLITE ANALYSIS OF A POPULATION OF UTAH PRAIRIE DOGS

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Prairie dogs (*Cynomys* sp.) have been the subject of numerous studies designed to better understand effects of social structure on genetic structure of populations. Recently, long-term behavioral studies have focused on understanding social structure and interactions within a colony of Utah prairie dogs (*C. parvidens*), which are threatened with extinction. We used 7 microsatellite loci to determine parentage for all juveniles in a Utah prairie dog colony born in 1996 and 1997. Parentage was determined for 50.3% of juveniles in 1996 and 45.3% of juveniles in 1997. Once parentage was determined, we looked at frequency of multiple paternity, male and female reproductive success, and inbreeding.

Key words: *Cynomys parvidens*, microsatellites, parentage, Utah prairie dogs

Because of their colonial nature and diurnal activity patterns, prairie dogs (*Cynomys* sp.) have been the subject of numerous studies designed to better understand effects of social structure on genetic structure within populations (Dobson et al. 1998; Hoogland 1995; Sugg et al. 1996; Travis et al. 1996). Most such studies have focused on black-tailed prairie dogs (*C. ludovicianus*---e.g., Chesser 1983a, 1983b; Dobson et al. 1998; Hoogland 1995). Recently, long-term behavioral studies have focused on understanding the social structure and interactions within a colony of threatened Utah prairie dogs (*C. parvidens*---J. L. Hoogland, in litt.).

In our study, observational and genetic data were used to address questions pertaining to parentage and social interactions in a colony of Utah prairie dogs. Utah prairie dogs are found in south-central Utah and are considered a threatened species (Hoffmann et al. 1993). The range of this species has contracted during the past several decades due to drought conditions and anthropogenic factors (Collier and Spillett 1975). Utah prairie dogs come into estrus once a year, both males and females usually mate with multiple partners, and litter size typically ranges from 1 to 6 (J. L. Hoogland, in litt.). This species copulates and gives birth underground, making observations of these events difficult.

The 1st objective of our study was to determine parentage for all juveniles born during 1996 and 1997 within a single colony. Parentage was determined using microsatellite analyses and exclusion and likelihood methodologies. Microsatellites are codominant markers that have increased in use over the past several years due to their high variability, ease of use, and repeatability. Microsatellites have been used to determine parentage, especially paternity, in a diverse array of animals including

swallows (*Hirundo rustica*---Primmer et al. 1995), chimpanzees (*Pan troglodytes*---Morin et al. 1994), horses (Marklund et al. 1994), canids (Binns et al. 1995), grizzly bears (*Ursus arctos*---Craighead et al. 1995), American bison (*Bison bison*---Mommens et al. 1998), armadillos (*Dasypus novemcinctus*---Prodohl et al. 1998), harbour seals (*Phoca vitulina*---Coltman et al. 1998), and rhesus macaques (*Macaca mulatta*---Kanthaswamy and Smith 1998). The 2nd objective of our study was to examine multiple paternity, reproductive success, and inbreeding in this colony.

MATERIALS AND METHODS

Behavioral data and blood were collected from every individual in a Utah prairie dog colony (Bryce Canyon National Park, Garfield County, Utah) for each year from 1996 through 2000. Methods of capture, blood sampling, and collection of behavioral data essentially follow that described by Hoogland (1995). Genetic analyses were performed on all adults and juveniles from samples of Utah prairie dogs collected in 1996 and 1997 (n = 147 and n = 225, respectively). Samples from 1996 and 1997 were selected because, at the time the study began, they were the only samples available for analyses.

Genomic DNA was extracted from about 50 µl of whole blood following the methods of Longmire et al. (1997). Seven microsatellite loci were amplified via polymerase chain reaction (PCR) with previously published primers developed by Stevens et al. (1997) for Columbian ground squirrels (*Spermophilus columbianus*). Although Stevens et al. (1997) reported 6 of 9 primer pairs amplified single loci in black-tailed prairie dogs, all primers were redesigned to allow multiplex gel loading (Table 1).

PCR amplifications were conducted in 15 µl volumes containing 50 ng of

genomic DNA, 10 pmols each primer, 9 µl True Allele Premix (Perkin Elmer Applied Biosystems, Foster City, California), and 3.8 µl of ddH_2O. The thermal profile consisted of a 12 min denaturation and enzyme activation cycle at 95°C; 10 cycles of 94°C for 15 s, 52°C for 60 s, and 72°C for 30 s; followed by 25 cycles of 89°C for 15 s, 55°C for 60 s, and 72°C for 30 s. A final 72°C incubation for 30 min was used to ensure that all reactions had gone to completion. For samples of DNA that did not amplify after repeated attempts using above temperatures, we tried 1 of the following: 1) original sample was redialyzed in 1 X TE (Tris, EDTA) for 2-3 days in an attempt to remove potential inhibitors, 2) original sample was reamplified by lowering the annealing temperature in the 10 cycle step from 52°C to 50°C. Variation at individual microsatellite loci was visualized using a Perkin-Elmer Applied Biosystems 377 Automated DNA Sequencer. Amplicons of each locus from a single individual were mixed (0.5 µl of each PCR product) and 1 µl of this mixture was combined with 3 µl of loading mix (2.5 µl of formamide, 0.5 µl of ROX size standard, 0.25 µl of loading buffer with blue dextran). The mixed PCR---loading mix solution was denatured at 95°C for 5 minutes and 1.5 µl was loaded into a single lane of a 5% polyacrylamide gel. All individuals were run for most loci at least twice to ensure accurate scoring of correct allele sizes. Genotypes were visualized using GENESCAN and GENOTYPER software.

Data Analysis

Observational data. -- Observational data were used to assign preliminary maternity and possible paternity for all juveniles in both years of the colony. Maternity was assigned behaviorally by capturing juveniles upon 1st emergence from their natal burrows. Pregnant females typically guard their burrows from all other females (J. L.

Hoogland, in litt.; see also, Hoogland 1995, 1997). Thus, maternity can be assigned to the female observed guarding and using the burrow from which juveniles 1st emerged. Potential paternity was assigned behaviorally to males seen displaying specific pre- and post-copulatory behaviors with the female guarding a particular burrow. Observational data were used to reduce the number of potential parents for parentage analysis.

Marker analysis. -- Unless otherwise mentioned, we used CERVUS 1.0 (Marshall et al. 1998) for all marker analyses, including computation of allele frequencies, expected and observed heterozygosity, frequency of null alleles, polymorphic information content (PIC---index of variability for each locus), deviations from Hardy-Weinberg Equilibrium, and 2 exclusion probabilities for parentage assignment for each locus separately and all 7 loci combined. Probability of identity (PI---probability of randomly selecting 2 individuals with identical genotypes) for each locus and for all variable loci was calculated as described by Paetkau and Strobeck (1994).

Maternity. -- Maternity was easily determined for both years of the study using behavioral data since each juvenile only had 1 potential mother. However, behavioral assignments were checked using exclusion and likelihood methodologies. Exclusion methods eliminate potential parents that have any mismatches with juveniles. Females were excluded as the actual mother of a juvenile if they had any mismatches that could not be explained due to null alleles. Exclusionary comparisons between candidate mothers and juveniles were done by sight of the investigator. Maternity was also analyzed using CERVUS. CERVUS utilizes allele frequencies, delta criterion, and likelihood methodologies. Each potential mother was assigned an LOD score (likelihood ratio---likelihood of the candidate mother and not a randomly chosen female being the

actual mother). Because there was only a single potential mother for each juvenile, LOD scores equaled ΔLOD scores (ΔLOD = LOD of the most-likely female minus LOD of the next most-likely female). Delta criterion were computed in simulation runs utilizing data specific parameters. The simulation model is described in Marshall et al. (1998). Number of simulation cycles was set at 100,000 to decrease variation in delta criterion. Proportion of loci mistyped was set at 0.03 to account for any errors such as mutations and null alleles. Proportion of loci typed was calculated separately for 1996 (0.935) and 1997 (0.925) using the marker analysis function of CERVUS. There were 42 adult females in 1996 and 46 adult females in 1997. All adult females for both years were sampled. Delta criterion were calculated at 95%, 80%, 65%, and 50% confidence levels.

Paternity. -- Paternity was assigned using exclusion and likelihood methodologies. Each method was initially used separately by considering only those males determined to be potential fathers based on observational data. Exclusion methods followed that used during maternity analyses. Paternity was assigned to the male that had no mismatches with the juvenile. For analyses using CERVUS, number of cycles, proportion of loci typed, proportion of loci mistyped, and confidence levels were the same as for maternity. There were 30 adult males in 1996 and 31 adult males in 1997. All adult males for both years were sampled. Each potential father was assigned an LOD score and the 2 most-likely fathers, those with the highest LOD scores, were used to calculate ΔLOD. Number of mismatches between juveniles and potential fathers and ΔLOD scores were considered when assigning paternity. The individual with the highest LOD score was assigned as the father of the juvenile.

Parentage. -- Only those females and males not excluded as potential parents in

maternity and paternity analyses were utilized in this analysis. For all analyses, adult females were considered the known parent and adult males were considered the candidate parent. Using exclusion methods, mother-juvenile dyads were compared to all potential fathers by sight of the investigator. Paternity was assigned only to adult males that did not have mismatches with the mother-juvenile dyad. Any mismatches within the tested triad resulted in removal of the male, not the female. Using likelihood methods, mother-juvenile dyads were compared to all potential fathers and paternity was assigned to the male with the highest LOD scores. Simulation parameters used for parentage analyses were the same as those used for paternity analyses. Final parentage was assigned to the male and female pair with the highest LOD scores and the fewest mismatches with the juvenile.

Multiple paternity. -- Frequency of multiple paternity was calculated by dividing the number of litters sired by 2 or males by the total number of litters that contained 2 or more offspring.

Reproductive success. -- Male reproductive success was calculated as the number of juveniles sired and female reproductive success was calculated as the number of juveniles per litter.

RESULTS

Based on behavioral observations, the 1996 colony was divided into 13 clans containing 42 adult females, 30 adult males, and 75 juveniles. All individuals in the population were genotyped for at least 1 locus. However, 3 juveniles (RSBSx2, H4x3, and BBx5) were only genotyped at 1, 2, and 2 loci, respectively. These 3 juveniles were removed from any subsequent paternity analyses, although maternity was assigned based

on behavioral observations. All remaining individuals in the population were genotyped for at least 5 loci.

Based on behavioral observations, the 1997 colony was divided into 14 clans containing 46 adult females, 31 adult males, and 148 juveniles. All individuals, except 2, were genotyped for at least 5 loci. Male 12 was only genotyped at 4 loci and juvenile 3SBSx5 was only genotyped at 3 loci. No individuals were removed from subsequent parentage analyses.

Markers. -- Numbers of alleles per locus ranged from 2-4 with a mean of 3.00 for both 1996 and 1997. Based on PIC and PI, loci GS08 and GS34 were most informative for both years (Table 2). Loci GS14 and GS17 were essentially fixed for a single allele (Tables 3 and 4) and locus GS20 was fixed for 1 allele. Locus GS12 was anomalous because all individuals were scored as heterozygotes. Loci GS12 and GS20 were removed from subsequent analyses. Exclusion probabilities decreased from 1996 to 1997 (Table 2), but only slightly with 1st-parent exclusionary power dropping from 42.3% to 41.2% and 2nd-parent exclusionary power dropping from 66.3% to 65.6%.

Maternity. -- Maternity was initially assessed for all 75 juveniles born in 1996. Three juveniles (RSBSx2, H4x3, and BBx5) were assigned maternity based only on behavioral observations, because of lack of genotypic data. However, maternity for juvenile BBx5 was supported with a "most-likely" confidence value using CERVUS. Based on exclusion methods, only 1 female was removed as potential mother. Female RSRAB was excluded as the mother of juvenile RSRABx4 due to a mismatch at locus GS34. In 4 other instances, mismatches apparently reflecting null alleles (a mutation preventing amplification and visualization of a gene product) prevented exclusion of a

potential mother. For the remaining 70 juveniles, no mismatches occurred and maternity was assigned to the female suggested by behavioral data.

Based on results from CERVUS, maternity was assigned at 95%, 80%, 65%, and 50% confidence levels 0, 11, 1, and 14 times, respectively. In an additional 44 instances, maternity was assigned at a "most-likely" confidence level. For the remaining 5 instances, maternity could not be assigned with any confidence because either negative LOD scores were assigned ($n = 4$) or the potential mother and the juvenile were genotyped at different loci ($n = 1$). For 3 instances in which a negative LOD was calculated, the potential mother had no mismatches with the juvenile and in the remaining instance the mother-juvenile dyad showed a mismatch explainable as a result of a null allele.

Maternity was also initially assessed for all 148 juveniles born in 1997. Based on exclusion methods, 2 females were removed as potential mothers for 2 juveniles. Female 3SBS was excluded as the mother of juvenile 3SBSx2 and female BB7 was excluded as the mother of juvenile BB7x1, both due to mismatches at locus GS34. In both instances, the dyads were supported with a "most-likely" confidence value by CERVUS. In 6 other instances, mismatches due to the apparent presence of null alleles prevented exclusion of a potential mother. In the remaining 140 instances, no mismatches occurred and maternity was assigned to the female suggested by observational data.

Based on results from CERVUS, maternity was assigned at 95%, 80%, 65%, and 50% confidence levels 7, 17, 4, and 9 times, respectively. In an additional 95 cases, maternity was assigned at a "most-likely" confidence level. The remaining 16 instances were all assigned negative LOD values, 12 of which were associated with no mismatches

between potential mothers and juveniles and 4 of which involved 1 mismatch explainable as a result of a null allele. In these 16 instances, exclusion methods designated the female as the most-likely mother.

Paternity. -- Paternity was assessed for 72 of the 75 juveniles born in 1996 with the 3 aforementioned juveniles being removed. Additionally, 8 juveniles (RSBSx1-5 and TSx1-3) had 1 potential father for which there was no blood sample.

Results from exclusion and likelihood approaches showed concordance for 58 of 72 (80.6%) juveniles. For 29 of these 58 juveniles, both methods chose the same male as the most-likely father. For the 29 remaining juveniles, both methods failed to exclude the same suite of males but no comparisons could be made because selection of a most-likely father was not possible with exclusion methods. Disagreements occurred for 14 juveniles for which 1 method excluded all males as candidate fathers and the other method failed to do so.

Paternity was assessed for all 148 juveniles born in the 1997 colony. One potential father (male 12) was not genotyped at loci GS08 or GS34 making exclusion of this male difficult when he was a potential father.

Results with exclusion and likelihood methods showed concordance for 126 of 148 (85.1%) juveniles. For 35 of these, both methods chose the same male as most-likely father. For 91 of these juveniles, both methods failed to excluded the same suite of males but selection of the most-likely father was not possible. For the remaining 22 juveniles, the 2 methods designated different males as most-likely fathers.

Parentage. -- Final maternity and paternity assignments (Appendices I, II) were made during parentage analyses. Mothers were assigned to all juveniles and only those

males not eliminated during exclusion analyses were used in parentage assignments. Based on likelihood analyses, more parentage assignments were made for both years than was predicted based on simulation calculations (Table 5). Complete parentage was assigned to 39 of 75 (52.0%) 1996 juveniles. Parentage was assigned at 95%, 80%, 65%, and 50% confidence levels to 0, 18, 6, and 6 juveniles, respectively. Delta LOD values ranged from less than 0.1 to 1.7 (Fig. 1). For an additional 9 juveniles, 5 were assigned at a "most-likely" confidence level and 4 were assigned negative LOD scores by CERVUS but were considered most-likely by exclusion methods. The remaining 36 juveniles had maternity assigned but either all potential fathers were excluded (n = 5) or paternity was ambiguous (n = 31), with 2 or more males as potential fathers.

Parentage was assigned to 67 of 148 (45.3%) 1997 juveniles. Parentage was assigned at 95%, 80%, 65%, and 50% confidence levels to 0, 16, 18, and 8 juveniles, respectively. Delta LOD values ranged from less than 0.1 to 2.0 (Fig. 2). In an additional 25 instances, 14 were assigned at the "most-likely" confidence level, 10 were assigned negative LOD scores but were considered most-likely by exclusion methods, and 1 was assigned a positive LOD score but was not indicated by CERVUS as most-likely. For the remaining 81 juveniles, maternity was assigned but either all potential fathers were excluded (n = 2) or paternity was ambiguous (n = 79), with 2 or more males as potential fathers.

Multiple paternity. -- In the 1996 population, a total of 19 litters encompassing all 75 juveniles could be determined. Only 1 litter contained a single juvenile, allowing us to test for multiple paternity in 18 litters. Eight of the 18 (44.4%) litters were determined to be multiply sired although, because paternity could not be determined for all offspring,

an additional 5 litters could still be multiply sired. Therefore, the actual range for the frequency of multiple paternity in the 1996 population lies somewhere between 44.4-72.2%. In the 1997 population, 37 litters encompassing all 148 juveniles could be determined. Thirty-five of 37 litters had 2 or more juveniles thus allowing us to test for multiple paternity. Fifteen of 35 (42.9%) litters were determined to be multiply sired although, an additional 18 litters still had the possibility of being multiply sired. Therefore, the actual frequency of multiple paternity in the 1997 population is between 42.9-94.3%.

Reproductive success. -- In 1996, the number of juveniles per litter ranged from 1-5 with a mean of 3.95. Only 19 of 42 (45.2%) adult or yearling females produced litters. In contrast, 37 of 46 (80.4%) adult or yearling females in 1997 produced litters. Litter size in 1997 ranged from 1-7 with a mean of 4.00.

There were a total of 55 different adult females over the 2 years of the study (Table 6). Thirteen of 55 (23.6%) were juveniles in 1996 and yearlings in 1997. Eight of 13 (61.5%) yearlings produced litters in 1997. The remaining 42 females were adults both years. For the 42 adult females, 11 (26.2%) produced litters both years, 26 (61.9%) produced litters only 1 year, and 5 (11.9%) did not produce litters either year. Nine of 42 (21.4%) adult females that were alive in 1996 did not survive until 1997.

Reproductive success for males was more difficult to determine due to difficulties in resolving paternity. Only 10 of 31 (32.3%) adult males in 1996 sired young. In 1996, paternity was resolved for 39 of 75 (52.0%) juveniles. Based on paternity for these 39 juveniles, number of juveniles sired per male ranged from 1-7 with a mean of 3.90. In 1997, 25 of 31 (80.6%) adult males were still considered potential fathers of 1 or more

juveniles. Paternity was resolved for 67 of 148 (45.3%) juveniles. Based on paternity for these 67 juveniles, number of juveniles sired per male ranged from 1-8 with a mean of 3.53.

There were 44 different adult males over 2 years (Table 7). Thirteen of 44 (29.5%) were juveniles in 1996 and yearlings in 1997. Seven of 13 (53.8%) yearlings sired young in 1997. The remaining 31 males were adults both years. For the 31 adult males, 6 (19.4%) sired young both years, 16 (51.6%) sired young only 1 year, and 9 (29.0%) did not sire young either year. Thirteen of the 31 (41.9%) adult males that were alive in 1996 did not survive until 1997.

DISCUSSION

Once parentage was assigned to juveniles in each year of the study, we investigated frequency of multiply paternity, male and female reproductive success, and inbreeding. Because parentage was assigned for 2 years in this colony, comparisons could be made between years in terms of levels of multiple paternity and reproductive success.

Multiple paternity. -- Multiple paternity was determined to have occurred in this study when offspring of the same litter were sired by 2 or more males. Multiple paternity is common among mammals that produce litters of multiple offspring (e.g., Hanken and Sherman 1981; Hoogland 1995; Robinson 1982). The frequency of multiple paternity calculated for both years of this population is an underestimate due to the inability to assign paternity to all juveniles. The calculated level of multiple paternity is essentially the same for both years of the study although the possible range is larger for the 1997 population. The range is larger in 1997 due to the fact that there were more cases of

unresolved paternity in 1997 than in 1996 (46.7% unresolved in 1996, 54.1% unresolved in 1997).

Reproductive success. -- Mean litter size and mean number of juveniles sired per male was essentially the same in both years of the study. There was a marked increase in the number of breeding males and females and juveniles born from 1996 to 1997. The increase in number of breeding adults is due to an increase in population size. The increase in breeding adults is reflected in an increase in juveniles born to the colony. It does appear that females were increasing the number of males with which they copulated. Mean number of males that each female copulated with in 1996 was 2.5 while mean number of males that each female copulated with in 1997 was 4.8.

Juvenile mortality. -- The survival rate for juveniles was low between 1996 and 1997. Only 26 of 75 (34.7%) juveniles in 1996 survived to be yearlings in 1997. The mortality rate in this population (65.3%) was above that reported for Gunnison's prairie dogs (50%---Hoogland 1998). The reason for the high mortality rate is unknown although this population did experience an outbreak of plague. Other possible reasons are a lack of resources including food, high rates of infanticide, and high rates of predator mortality.

Inbreeding. -- Potential incidences of inbreeding in this population could be determined in those instances when individuals born in 1996 bred with relatives during 1997. We do not know the relationships of those individuals that were breeding adults or yearlings in 1996. Therefore, our estimates of inbreeding are probably lower than the actual level in the colony.

Six instances of potential inbreeding apparently occurred in the 1997 breeding

season (Table 8). In 2 instances, a son from 1996 was a potential father of at least 1 juvenile in his mother's 1997 litter. In 2 other instances, potential father-daughter mating occurred. In both instances a potential father of a 1996 juvenile female was a potential father of 1 or more juveniles of that female's 1997 litter. In 1 litter there was a potential half-sibling mating in which, a half-brother was a possible father of all of his half-sister's offspring. In the remaining example, inbreeding was confirmed because the potential father of all juveniles in a litter was either the female's father or 1 of 2 brothers. While individuals do appear to be copulating with close relatives, we are unable to confirm actual inbreeding in all but 1 instance because of ambiguous paternity.

Despite some success with parentage analyses, we were unable to assign parentage for all juveniles in the population. The 1st difficulty encountered in this study was the lack of genetic variation at several loci. Three loci were fixed (GS20) or nearly fixed (GS14, GS17) for 1 allele. It is unclear whether low variability is characteristic of the species or of the population we studied. These loci are variable in other sciurids including black-tailed prairie dogs (Stevens et al. 1997) and Gunnison's prairie dogs (Haynie et al., in prep.). Additionally, locus GS12 was scored as a heterozygote for every individual in the population for both years. The reason for this result is unknown and such a phenomenon has not been reported in other studies. For this reason, locus GS12 was removed from the study. For this population, only loci GS08, GS26, and GS34 were informative.

Because of low levels of genetic variability, the exclusion probabilities we used (PE1 = 41.2-42.3%, PE2 = 65.7-66.3%) were lower than typical in parentage studies (Coltman et al. 1998; Kanthaswamy and Smith 1998; Mommens et al. 1998; Primmer et

al. 1995) including other prairie dog species (Haynie et al., in prep.).

Another problem faced when assigning parentage was the degree of relatedness among candidate parents. This problem cannot be addressed in the absence of a pedigree for adults in the population. However, the development of a pedigree should aid in resolving relationships between potential parents and among potential parents and offspring.

Mutations are another difficulty. An undetected mutation may cause exclusion of a parent. Mutation rates can be calculated by dividing the total number of mismatches between known mother-juvenile dyads by the total number of alleles at a locus. Mutation rates are calculated for each locus separately. Suspected mutations were found at locus GS34 in both years of this study. When potential null alleles were not considered, the mutation rate at this locus was 0.005-0.007. When such alleles were considered, the mutation rate increased by an order of magnitude (0.023-0.069). Suspected null mutations were also found at loci GS08 and GS26 where mutation rates were 0.007 and 0.002, respectively. The mutation rates we detected were within the range expected for microsatellites (Weber and Wong 1993).

Despite the problems listed above, we were able to assign parentage to 52.0% of 1996 juveniles and 45.3% of 1997 juveniles. Most parentage studies result in only a fraction of juveniles being assigned parentage even when large behavioral data sets and highly variable loci are combined. Kanthaswamy and Smith (1998) were able to assign parentage to 98.4% of rhesus macaque juveniles, but most studies report markedly lower success rates (e.g., Coltman et al. 1998; Keane et al. 1997; Petri et al. 1997). Despite the often low rate of success, parentage studies do provide more clarity than can be obtained

by observational data alone.

To address the questions and problems raised by this study, a pedigree will be developed using the information from this study. The pedigree will be extended with genotypes for individuals collected from 1998-2000 using loci GS08, GS26, and GS34. In addition, other nuclear markers are being tested to determine if any will provide further resolution in paternity assignments. The development of an extensive pedigree should contribute to addressing questions pertaining to multiple paternity, reproductive success, and relatedness of interacting individuals. Additionally, it is our intention to sample other populations of Utah prairie dogs to determine if they too exhibit low variability at these microsatellite loci.

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Table 1. -- Microsatellite loci and primers (6FAM = blue label, NED = yellow label, HEX = green label)

GS26 2F2R:	NED-GGCTCCAAGTCCCAGGGAC
	GTGTCTTGGTCCTCATCCTGCCAATTTC
GS08 2F4R:	HEX-ACCAATGGGAGAGCACATCCAA
	GTGTCTTAAACTCCTTGTAATAGCCCCCTG
GS20 1F1R:	6FAM-GCCCAGCCATCACCCTCACC
	GTGTCTTTCCAGAGTTTTTCAGACACA
GS17 1F1R:	6FAM-CAATTCGTGGTGGTTATATC
	GTGTCTTCTGTCACCTATATGAACACA
GS12 1F1R:	NED-CCAAGAGAGGCAGTCGTCCAG
	GTGTCTTTCGAGCAGAGCACTTCACAGA
GS14 2F3R:	6FAM-CAGAATCAGGTGGGTCCATAGTG
	GTGTCTTGATGAAACCTATTTGCCTTCCTTC
GS34 1F1R:	NED-CTTTCTTCTGCTCTGTTATC
	GTGTCTTCACCTCACTTTATCTCTGAA

Table 2. -- Locus name and descriptive statistics for genetic variation at each locus for a population of Utah prairie dogs (*Cynomys parvidens*) collected from Bryce Canyon National Park, Garfield County, Utah, 1996-1997. A = number of alleles, n = sample size, H_O = observed heterozygosity, H_E = expected heterozygosity, PIC = polymorphism information content, PE1 and PE2 are 1st- and 2nd-parent exclusionary probabilities, respectively, and PI = probability of identity.

	1996								1997							
Locus[a]	A	n	H_O	H_E	PIC	PE1	PE2	PI[b]	A	n	H_O	H_E	PIC	PE1	PE2	PI[b]
GS08	4	145	0.369	0.647	0.574	0.211	0.359	0.197	4	222	0.342	0.630	0.557	0.199	0.343	0.210
GS14	3	145	0.014	0.014	0.014	0.000	0.007	0.973	3	225	0.009	0.009	0.009	0.000	0.004	0.983
GS17	2	145	0.007	0.007	0.007	0.000	0.003	0.987	2	225	0.004	0.004	0.004	0.000	0.002	0.991
GS26	2	145	0.386	0.401	0.320	0.080	0.160	0.440	2	224	0.317	0.364	0.297	0.006	0.149	0.471
GS34	4	111	0.441	0.618	0.562	0.205	0.367	0.200	4	145	0.441	0.627	0.576	0.214	0.381	0.190
Mean	3.0		0.243	0.339	0.295	0.423	0.663	7.7×10^{-5}	3.0		0.223	0.327	0.155	0.412	0.656	1.8×10^{-2}

[a]Locus names as those originally described by Stevens et al. (1997).

[b]Probability of Identity calculated following the method of Paetkau and Strobeck (1994).

Table 3. -- Allele frequencies for 147 *Cynomys parvidens* from Bryce Canyon National Park, Garfield County, Utah, 1996. For each locus, the allele, how many times that allele was found in the population, number of heterozygous individuals with that allele, number of homozygous individuals with that allele, and allele frequency are reported.

Locus	Allele	Count	Heterozygotes	Homozygotes	Frequency
GS08	183	85	33	26	0.3014
	185	129	41	44	0.4574
	189	2	2	0	0.0071
	191	66	28	19	0.2340
GS14	183	1	1	0	0.0034
	187	1	1	0	0.0034
	193	288	2	143	0.9931
GS17	151	1	1	0	0.0034
	153	289	1	144	0.9966
GS26	105	210	56	77	0.7241
	111	80	56	12	0.2759
GS34	319	18	18	0	0.0811
	323	122	38	42	0.5495
	325	27	3	12	0.1216
	327	55	39	8	0.2477

Table 4. -- Allele frequencies for 225 *Cynomys parvidens* from Bryce Canyon National Park, Garfield County, Utah, 1997. For each locus, the allele, how many times that allele was found in the population, number of heterozygous individuals with that allele, number of homozygous individuals with that allele, and allele frequency are reported.

Locus	Allele	Count	Heterozygotes	Homozygotes	Frequency
GS08	183	138	52	43	0.3108
	185	214	64	75	0.4820
	189	1	1	0	0.0023
	191	91	35	28	0.2050
GS14	183	1	1	0	0.0022
	187	1	1	0	0.0022
	193	448	2	223	0.9956
GS17	151	1	1	0	0.0022
	153	449	1	224	0.9978
GS26	105	341	71	135	0.7612
	111	107	71	18	0.2388
GS34	319	40	28	6	0.1379
	323	158	52	53	0.5448
	325	27	5	11	0.0931
	327	65	43	11	0.2241

Table 5. -- Confidence levels, critical ΔLOD scores (ΔLOD = LOD of most-likely male minus LOD of next most-likely male), and percentage of predicted and assigned parentage for 75 and 148 juvenile *Cynomys parvidens* from Bryce Canyon National Park, Garfield County, Utah, 1996 and 1997, respectively. Calculations were made using CERVUS 1.0 (Marshall et al. 1998).

Confidence levels	Critical LOD	Predicted assignments	Assignments made
1996			
95%	2.20	0%	0%
80%	1.06	1%	24%
65%	0.80	3%	32%
50%	0.58	7%	40%
1997			
95%	2.19	0%	0%
80%	1.28	0%	11%
65%	0.85	2%	25%
50%	0.60	5%	32%

Table 6. -- Survival and reproduction of adult female *Cynomys parvidens* from Bryce Canyon National Park, Garfield County, Utah, 1996-1997. All females that were either adults in 1996 and 1997 and produced litters in 1996 and/or 1997 are listed. The female's 1996 and 1997 markings are recorded, as well as number of juveniles, if any, she had. A dash (--) indicates that a female did not have any juveniles during a particular year or did not survive until that year. Females that were juveniles in 1996 were listed only if they survived to be yearlings in 1997.

1996 marking	# juveniles	1997 marking	# juveniles
RSBS	5	RSBS	4
TS	3	HTS	4
C7	5	H7	4
FO	5	C2	6
5SRS	2	--	--
RAC	4	RAC	1
BB4	4	--	--
57	5	--	--
76	3	76	6
3SBS	5	3SBS	7
H4	3	--	--
RSRAB	4	--	--
RR9	4	--	--
C6	5	--	--
RS	4	RS	5

Table 6. Continued.

1996 marking	# juveniles	1997 marking	# juveniles
BB5	5	BB5	4
5str	1	5str	7
HBS	5	HBS	--
HRS	4	HRS	4
BS	--	BS	4
WA	--	--	--
56	--	56	3
RAB	--	RAB	3
BB7	--	BB7	5
H2	--	H2	4
70	--	70	3
H	--	WA4	1
3SRS	--	3SRS	--
RB3	--	RB3	--
2RS	--	2RS	4
51	--	51	5
BB2	--	BB2	4
4str	--	4str	4
BSBB	--	--	--
CR	--	CR	--
4SBS	--	4SBS	2

Table 6. Continued.

1996 marking	# juveniles	1997 marking	# juveniles
BB6	--	BB6	4
FR	--	FR	5
CO	--	CO	3
60	--	60	4
CBB	--	CBB	4
WA6	--	WA6	2
H4x3	--	WA3	3
BBx3	--	55	3
RCx3	--	77	4
CRx4	--	WARS	5
RSx3	--	RRO	5
5SRSx2	--	H6	4
HRSx3	--	62	4
HRSx1	--	4SRS	4
4strx3	--	H5	--
3SBSx4	--	F	--
RSBSx4	--	WA2	--
BBx2	--	C4	--
HRSx2	--	WA7	--

Table 7. -- Survival and reproduction of adult male *Cynomys parvidens* from Bryce Canyon National Park, Garfield County, Utah, 1996-1997. All males that were either adults in 1996 and 1997 and sired young in 1996 and/or 1997 are listed. The male's 1996 and 1997 markings are recorded, as well as number of juveniles, if any, actually sired. The number in parentheses is the number of additional juveniles for which the male is still considered a potential father. A dash (--) indicates that a male did not sire juveniles during a particular year or did not survive until that year. Males that were juveniles in 1996 were listed only if they survived to be yearlings in 1997.

1996 marking	# juveniles	1997 marking	# juveniles
2	6	2	4(6)
21	3(4)	21	5(18)
4	6(4)	--	--
20	1	20	(2)
5	(6)	--	--
02	1(8)	--	--
R01	4(19)	--	--
6	7(19)	6	3(16)
8	7(8)	8	2(4)
14	4(2)	14	4(15)
31	--	--	--
3	--	3	1(17)
44	--	44	3(2)
R16	--	--	--

Table 7. Continued.

1996 marking	# juveniles	1997 marking	# juveniles
R22	--	04	4(7)
R44	--	16	8(4)
R31	--	46	3(3)
R12	--	--	--
R14	--	22	4(10)
R23	--	--	--
R42	--	--	--
15	--	--	--
17	--	--	--
R10	--	--	--
R13	--	19	2(6)
R17	--	7	1(5)
R21	--	47	(5)
R00	--	00	2(3)
26	--	--	--
R41	--	30	3(7)
Unknown	--	12	(14)
3SBSx1	--	R12	(7)
C7x3	--	R44	(2)
RSx2	--	25	3(16)
RSx1	--	9	7(15)

Table 7. Continued.

1996 marking	# juveniles	1997 marking	# juveniles
RCx1	--	13	5(5)
CRx1	--	R01	(7)
BBx1	--	37	2
CBx2	--	R41	--
RSBSx1	--	R14	--
FOx1	--	R16	--
CRx2	--	R23	--
CRx3	--	R34	--
4strx1	--	()	--

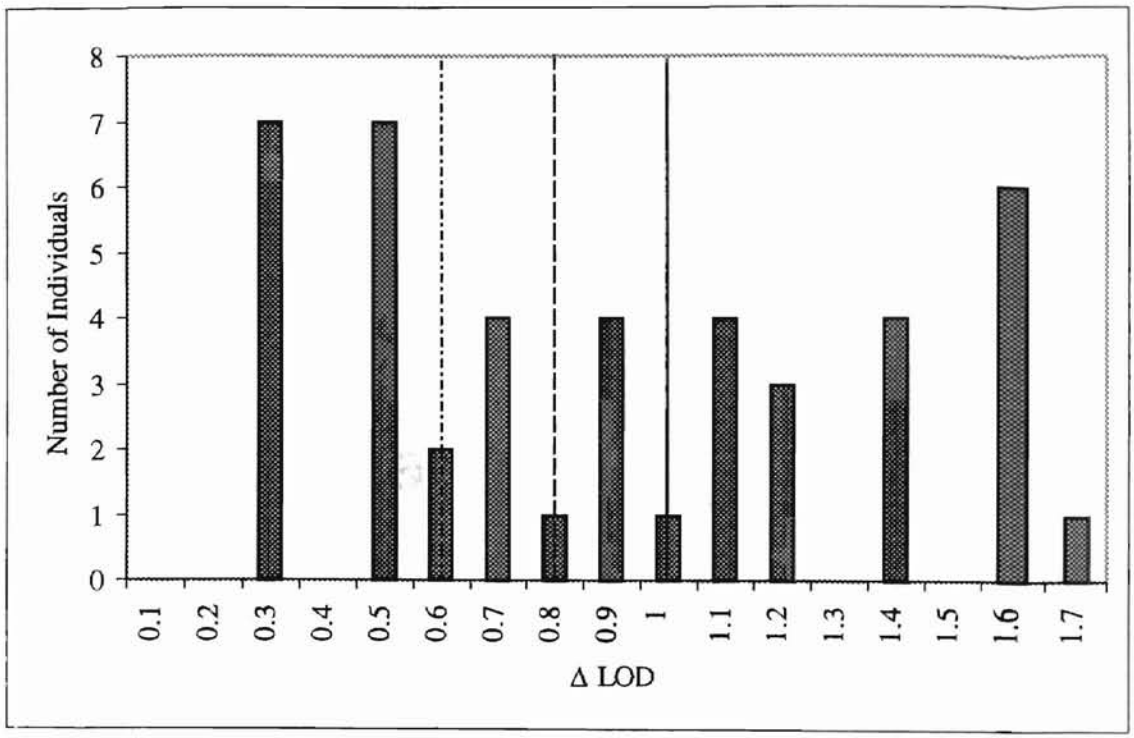
Table 8. -- Potential occurrences of inbreeding in a population of *Cynomys parvidens* from Bryce Canyon National Park, Garfield County, Utah, 1997. The female and male between which inbreeding potentially occurred, their relationship, and the number of juveniles they potentially produced together are listed. The parentheses under the male and female headings contain the marking for the individual in 1996. The parentheses under the potential number of juveniles heading are the markings of the juveniles which were potentially produced by this pair.

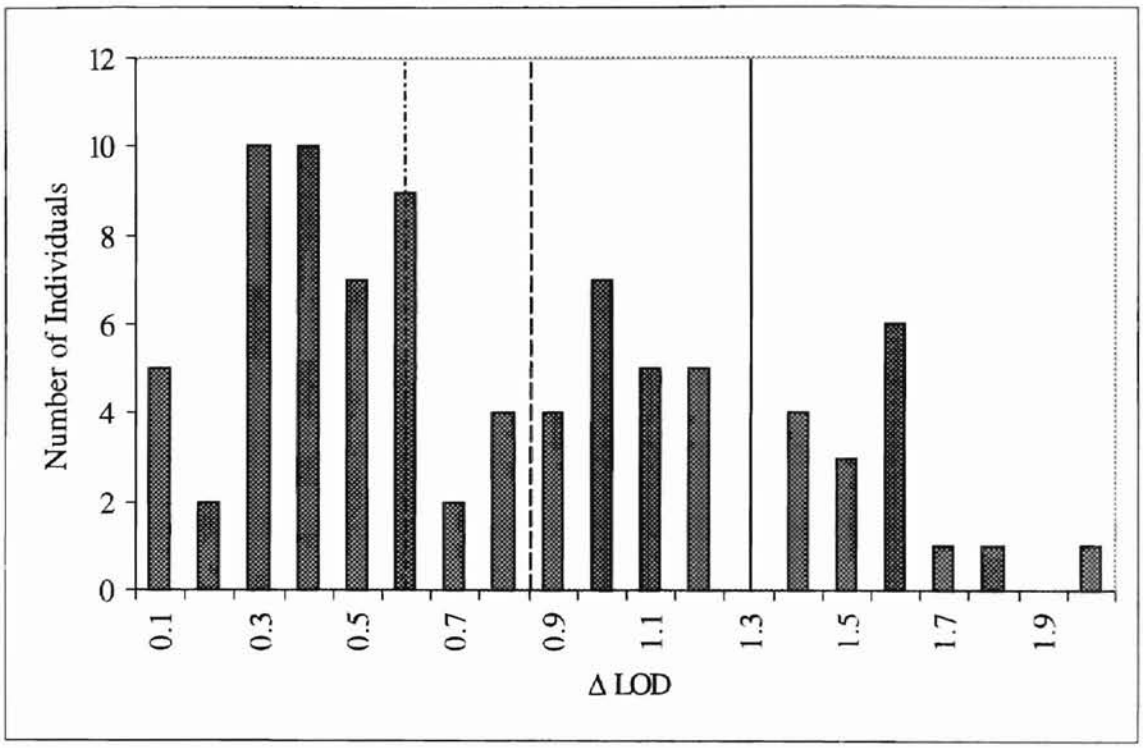
Female	Male	Relationship	Potential # of juveniles
H7(C7)	R44(C7x3)	mother-son	1(H7x3)
RS(RS)	25(RSx2)	mother-son	2(RSx1, RSx3)
77(RCx3	8(8)	daughter-father	4(F7x1-4)
62(HRSx3)	14(14)	daughter-father	2(2x2-3)
WARS(CRx4)	R01(CRx10	half-siblings	5(WARSx1-5)
RRO(RSx3)	14(14)	daughter-father	5(RROx1-5)
RRO(RSx3)	9(RSx1)	siblings	5(RROx1-5)
RRO(RSx3)	25(RSx2)	siblings	5(RROx1-5)

FIGURE LEGENDS

Fig. 1. -- Distribution of ΔLOD scores for most-likely candidate males calculated during parentage assignments for 75 juvenile *Cynomys parvidens* from Bryce Canyon National Park, Garfield County, Utah, 1996. Two individuals with ΔLOD scores < 0.1 are not included. Critical ΔLOD for 80, 65, and 50% confidence levels are shown with solid, dashed, and dotted lines, respectively. No parentage assignments were made at the 95% confidence level. Calculations were made using CERVUS 1.0 (Marshall et al. 1998).

Fig. 2. -- Distribution of ΔLOD scores for most-likely candidate males calculated during parentage assignments for 148 juvenile *Cynomys parvidens* from Bryce Canyon National Park, Garfield County, Utah, 1997. Fourteen individuals with ΔLOD scores < 0.1 are not included. Critical ΔLOD for 80, 65, and 50% confidence levels are shown with solid, dashed, and dotted lines, respectively. No parentage assignments were made at the 95% confidence level. Calculations were made using CERVUS 1.0 (Marshall et al. 1998).


Number of Individuals
0
1
2
3
4
5
6
7
8
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
Δ LOD


Number of Individuals
12
10
8
6
4
2
0
0.1
0.3
0.5
0.7
0.9
1.1
1.3
1.5
1.7
1.9
Δ LOD

APPENDIX I

Parentage of 1996 colony. -- Parentage was determined using observational, exclusion, and likelihood methods for 75 *Cynomys parvidens* juveniles from Bryce Canyon National Park, Garfield County, Utah. Maternity (female ID) and paternity (male ID) are final parentage assignments for this population. All confidence levels reported were calculated by CERVUS and represent the confidence of the mother-juvenile-father triad. A dash (--) indicates that no mother, father, or confidence level could be assigned. An asterisk (*) beside a juvenile ID indicates that 1 potential father was not sampled. An ml indicates a "most-likely" confidence level. A (-LOD) under the confidence level column indicates that the individuals selected as the parents had a "most-likely" confidence level based on exclusion methods but could not be assigned a confidence level using CERVUS.

Juvenile ID	Female ID	Male ID	Confidence level
RSBSx1*	RSBS	2	80%
RSBSx2*	RSBS	2	--
RSBSx3*	RSBS	2	80%
RSBSx4*	RSBS	2	80%
RSBSx5*	RSBS	2	80%
TSx1*	TS	--	--
TSx2*	TS	--	--
TSx3*	TS	2	65%
C7x1	C7	21	50%
C7x2	C7	4	80%

APPENDIX I. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
C7x3	C7	20	50%
C7x4	C7	4	80%
FOx1	FO	5,02	--
FOx2	FO	5,02	--
FOx3	FO	5,02	--
FOx4	FO	5,02	--
FOx5	FO	5,02	--
5SRSx1	5SRS	2	80%
5SRSx2	5SRS	5,02	--
RCx1	RC	R01,6	--
RCx2	RC	R01,6	--
RCx3	RC	8,R01,6	--
RCx4	RC	R01,6	--
BB4x1	BB4	6	50%
BB4x2	BB4	6	ml
BB4x3	BB4	6	ml
BB4x4	BB4	6	50%
CRx1	57	8	65%
CRx2	57	6	50%
CRx3	57	8	80%
CRx4	57	6	80%

APPENDIX I. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
CRx5	57	6	80%
RABx1	76	8	80%
RABx2	76	8	80%
RABx3	76	R01,6	--
3SBSx1	3SBS	8,R01,6	--
3SBSx2	3SBS	8	65%
3SBSx3	3SBS	R01,6	--
3SBSx4	3SBS	R01,6	--
3SBSx5	3SBS	8	65%
H4x1	H4	8,R01,6	--
H4x2	H4	R01,6	--
H4x3	H4	--	--
RSRABx1	RSRAB	R01,6	--
RSRABx2	RSRAB	R01,6	--
RSRABx3	RSRAB	R01,6	--
RSRABx4	RSRAB	R01,6	--
4strx1	RR9	R01	ml
4strx2	RR9	R01	ml
4strx3	RR9	R01	--(-LOD)
4strx4	RR9	R01	--(-LOD)
CBx1	C6	8,R01,6	--

APPENDIX I. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
CBx2	C6	8,R01,6	--
CBx3	C6	R01,6,8	--
CBx4	C6	R01,6	--
CBx5	C6	R01,6	--
RSx1	RS	14	80%
RSx2	RS	14	80%
RSx3	RS	14	80%
RSx4	RS	14	65%
BBx1	BB5	4,21	--
BBx2	BB5	4	80%
BBx3	BB5	4	80%
BBx4	BB5	4	80%
BBx5	BB5	--	--
5strx1	5str	21,4,14	--
WAx1	HBS	21	--(-LOD)
WAx2	HBS	4	50%
WAx3	HBS	21,4	--
WAx4	HBS	4,21	--
WAx5	HBS	21	--(-LOD)
HRSx1	HRS	02	65%
HRSx2	HRS	02,8	--

APPENDIX I. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
HRSx3	HRS	14,02,8	--
HRSx4	HRS	8	ml

APPENDIX II

Parentage of 1997 colony. -- Parentage was determined using observational, exclusion, and likelihood methods for 148 *Cynomys parvidens* juveniles from Bryce Canyon National Park, Garfield County, Utah. Maternity (female ID) and paternity (male ID) are final parentage assignments for this population. All confidence levels reported were calculated by CERVUS and represent the confidence of the mother-juvenile-father triad. A dash (--) indicates that no mother, father, or confidence level could be assigned. An ml indicates a "most-likely" confidence level. A (-LOD) under the confidence level column indicates that the individuals selected as the parents had a "most-likely" confidence level based on exclusion methods but could not be assigned a confidence level using CERVUS.

Juvenile ID	Female ID	Male ID	Confidence level
FRx1	FR	7,47	--
FRx2	FR	7,47	--
FRx3	FR	00	50%
FRx4	FR	00	--(+LOD)
FRx5	FR	7	ml
COx1	CO	47,7	--
COx2	CO	00,7,47,20	--
COx3	CO	00,7,47,20	--
HRBx1	51	16	50%
HRBx2	51	16,19,04	--
HRBx3	51	19	65%

APPENDIX II. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
HRBx4	51	19	--(-LOD)
HRBx5	51	19,04,16	--
Fx1	76	16	80%
Fx2	76	04	50%
Fx3	76	16	ml
Fx4	76	16	--(-LOD)
Fx5	76	16	ml
Fx6	76	16	--(-LOD)
3SBSx1	3SBS	04	ml
3SBSx2	3SBS	16	65%
3SBSx3	3SBS	44	50%
3SBSx4	3SBS	04,19	--
3SBSx5	3SBS	16,44,04,19	--
3SBSx6	3SBS	16	50%
3SBSx7	3SBS	04	ml
WA3x1	WA3	04	ml
WA3x2	WA3	16,04	--
WA3x3	WA3	04	80%
BB2x1	BB2	04,19	--
BB2x2	BB2	44	65%
BB2x3	BB2	44	65%

APPENDIX II. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
BB2x4	BB2	04,19	--
RSBSx1	RSBS	2	--(-LOD)
RSBSx2	RSBS	2	80%
RSBSx3	RSBS	2	80%
RSBSx4	RSBS	2	--(-LOD)
HTSx1	HTS	2,12	--
HTSx2	HTS	12,2	--
HTSx3	HTS	2,12	--
HTSx4	HTS	2,12	--
WA4x1	WA4	3,00.21	--
C2x1	C2	30,12	--
C2x2	C2	30,12	--
C2x3	C2	30	80%
C2x4	C2	30	80%
C2x5	C2	30,12	--
C2x6	C2	30	65%
F5x1	55	14	80%
F5x2	55	3,21,6	--
F5x3	55	3,21,6	--
0x1	60	3,21,6	--
0x2	60	14	65%

APPENDIX II. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
0x3	60	14,3,21,6	--
0x4	60	3,21,6	--
H7x1	H7	6,3,21	--
H7x2	H7	6	65%
H7x3	H7	3,21,R44,6	--
H7x4	H7	6,3,21	--
2RSx1	2RS	3,21	--
2RSx2	2RS	14,3,21	--
2RSx3	2RS	3,21	--
2RSx4	2RS	3,21	--
4strx1	4str	25,9,22	--
4strx2	4str	25	--(-LOD)
4strx3	4str	25,9,22	--
4strx4	4str	25	80%
HOx1	5str	21	50%
HOx2	5str	13	80%
HOx3	5str	21	65%
HOx4	5str	21	65%
HOx5	5str	13	--(-LOD)
HOx6	5str	13	80%
HOx7	5str	21	65%

APPENDIX II. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
WAx1	WA6	21,6	--
WAx2	WA6	21,6	--
BB5x1	BB5	14,3,21,6	--
BB5x2	BB5	14,3,21,6	--
BB5x3	BB5	14	80%
BB5x4	BB5	3,21,6	--
CBBx1	CBB	21	50%
CBBx2	CBB	14,21,6	--
CBBx3	CBB	6	65%
CBBx4	CBB	6	65%
BSBBx1	70	3,6	--
BSBBx2	70	3	--
BSBBx3	70	6,14,3	--
F7x1	77	8,R01,R12	--
F7x2	77	8,R01	--
F7x3	77	8	50%
F7x4	77	8	ml
RACx1	RC(RAC)	R12,R01,44,8	--
WARSx1	WARS	R01,R12	--
WARSx2	WARS	R12,R01	--
WARSx3	WARS	R12,8	--

APPENDIX II. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
WARSx4	WARS	R12,R01	--
WARSx5	WARS	R12,R01	--
BBx1	BB6	46,22	--
BBx2	BB6	46,22	--
BBx3	BB6	46	65%
BBx4	BB6	46	80%
H2x1	H2	9	65%
H2x2	H2	9.22	--
H2x3	H2	9	65%
H2x4	H2	--	--
3x1	4SBS	13,22,46	--
4SBSx1	4SBS	46	80%
RROx1	RRO	25,14,9	--
RROx2	RRO	9,25,14	--
RROx3	RRO	25,14,9	--
RROx4	RRO	9,14,25	--
RROx5	RRO	25,14,9	--
BSx1	BS	--	--
BSx2	BS	12,2	--
BSx3	BS	12,2	--
BSx4	BS	12,2	--

APPENDIX II. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
H6x1	H6	30,12	--
H6x2	H6	30,12	--
H6x3	H6	30,12	--
H6x4	H6	30,12	--
TSx1	56	22,13	--
TSx2	56	22,13	--
TSx3	56	13,22	--
BB7x1	BB7	13	--(-LOD)
BB7x2	BB7	9,25	--
BB7x3	BB7	9,25	--
BB7x4	BB7	22	ml
BB7x5	BB7	22,13	--
RABx1	RAB	9,25	--
RABx2	RAB	22	80%
RABx3	RAB	22	ml
2x1	62	13	80%
2x2	62	14,9,25	--
2x3	62	14,9,25	--
2x4	62	9	80%
RSx1	RS	25,14	--
RSx2	RS	37	65%

APPENDIX II. Continued.

Juvenile ID	Female ID	Male ID	Confidence level
RSx3	RS	25,14	--
RSx4	RS	14	ml
RSx5	RS	37	65%
HRSx1	HRS	9	ml
HRSx2	HRS	9	--(-LOD)
HRSx3	HRS	9	--(-LOD)
HRSx4	HRS	9	--(-LOD)
4SRSx1	4SRS	22	ml
4SRSx2	4SRS	25	ml
4SRSx3	4SRS	25,9	--
4SRSx4	4SRS	9,25	--

CHAPTER IV

SUMMARY

Despite the presence of large behavioral data sets and highly variable loci, parentage could not be assigned to all juveniles in either study colony. Nor could high levels of confidence be associated with all parentage assignments made. However, most parentage studies result in only a portion of juveniles with parentage assigned (e.g., Coltman et al. 1998; Kanthaswamy and Smith 1998; Keane et al. 1997; Petri et al. 1997; Prodohl et al. 1998) and confidence levels of 50% generally are above those obtained with behavioral data alone. Parentage was assigned to 31.0% of Gunnison's prairie dog (*Cynomys gunnisoni*) juveniles and 52.0% and 45.3% of Utah prairie dog (*C. parvidens*) juveniles from 1996 and 1997, respectively. Even though parentage could not be assigned to all juveniles, enough assignments were made in both studies to begin addressing several key issues in behavioral ecology.

Multiple paternity, male and female reproductive success, and inbreeding were addressed in chapters 2 and 3. The frequencies of multiple paternity we found in the Gunnison's and Utah prairie dog colonies, respectively, 27.1% and 42.9-44.4% were markedly higher than the 5% reported for black-tailed prairie dogs (*C. ludovicianus*---Hoogland 1995), but lower than the 89% reported for California ground squirrels (*Spermophilus beecheyi*---Boellstorff et al. 1994). For both of our study colonies, the actual frequency is probably higher than reported because paternity was unresolved for a number of juveniles.

Instances of potential inbreeding were documented only in the Utah colony. In that colony, inbreeding apparently involved combinations of mothers and sons, fathers

and daughters, half-siblings, and full-siblings. Although inbreeding avoidance has been hypothesized for some species of prairie dogs (Hoogland 1995), it appears to be common in the Utah colony. Inbreeding generally is viewed as maladaptive (Brown and Brown 1998; Keller 1998; Ralls et al. 1986), although Chesser and Ryman (1986) and Margulis (1998a, 1998b) proposed that inbreeding may be advantageous under certain circumstances including instances where the cost of migrating to find unrelated mates outweighs the cost of inbreeding.

A variety of difficulties deterred from the assignment of parentage in both colonies we examined. For the Gunnison's prairie dogs, locus GS22 was removed in several instances because of scoring difficulty. Additionally, several potential mothers were genotyped at 4 or fewer loci. Determination of parentage for 134 juveniles was confounded by lack of genetic data (e.g., a female genotyped at 4 or less loci) for 1 or more potential mothers. The reason that adult females were so difficult to genotype is unclear. All samples from adult females were extracted under the same conditions as juveniles and adult males. All samples from all individuals were also run under the same PCR and gel conditions. Only 2 adult males presented the same difficulty. The main difficulty in the Utah prairie dog study was lack of genetic variation. Only 3 loci, GS08, GS34, and GS26, were variable and informative in terms of parentage assignment. This lack of genetic variability posed a problem in terms of resolving paternity and in several instances resulted in no males being excluded as potential parents.

To address questions and problems raised by both studies, extended pedigrees need to be constructed by genotyping the 1991-1993 Gunnison's prairie dog samples and the 1998-2000 Utah prairie dog samples and adding that data to the data already gathered

in this study. Additionally, other nuclear markers need to be located that will aid in resolving parentage assignments. The development of mitochondrial markers may also add clarity to maternity assignments for Gunnison's prairie dogs. Also, other Utah prairie dog populations need to be sampled to determine if they too lack genetic variation at the loci used in this study.

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